BIOPROSPECTING FOR HYPOGLYCEMIC ACTIVITIES AND SAFETY OF SELECTED TRADITIONALLY USED PLANTS IN THE MANAGEMENT OF DIABETES MELLITUS

By

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DECLARATION

I, Makori Wycliffe Arika, 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

This thesis is dedicated to my father Makori Nyang'ute Harrison, my mother Damaris Moraa Makori and my daughter Shanne Sky Moraa Arika from whom I derive my inspiration.

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ABBREVIATIONS AND ACRONYMS

µL	Microlitre
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
Be	Beryllium
BUN	Blood urea nitrogen
CK	Creatine kinase
DLC	Differential leukocyte count
g	gram
g/dl	grams per decilitre
GAD	Glutamic acid decarboxylase
Glc	Glucose
Hb Alc	Glycosylated hemoglobin
Hb	Hemoglobin
HCl	Hydrochloric acid
IAA	Insulin autoantibodies
ICA	Islet cell autoantibodies
kg	kilogram
L	Liter
LDH	Lactate dehydrogenase
MCHC	Mean cell heamoglobin concentration
Mg	Magnesium
mg	milligram
ml	milliliter
Mn	Manganese
PCV	Packed cell volume
Ppg	Post prandial glucose
RBC	Red blood cell
SD	Standard deviation
SEM	Standard error of mean
Si	Silicon
TNF	Tumor necrosis factor
TXRF	Total x-ray reflection fluorescence
WBC	White blood cell
WHO	World health organization

ABSTRACT

Diabetes mellitus is a group of metabolic diseases characterized by high blood sugar levels that result from defects in insulin secretion, or action, or both. The disorder completely throws the metabolism of dietary carbohydrates, lipids and proteins into disarray. This condition in its fully developed form is characterized by high blood sugar level (hyperglycemia), glycosuria, increased protein breakdown, ketosis and acidosis. Diabetes is a chronic medical condition which can be controlled but lasts a lifetime. Conventional management of diabetes mellitus is expensive and therefore unaffordable and sometimes unavailable to many patients especially in developing and underdeveloped nations. Such antidiabetic drugs have been found to have side effects with long term use and hence facilitating the continued usage of herbal prescriptions as an alternative way to compliment orthodox pharmacotherapy. However, there is limited scientific evidence regarding safety and efficacy to back up the continued therapeutic application of herbal remedies. The aim of this study was to determine through bioassay-guided screening, efficacy and toxic components present in five selected medicinal plants. The following herbs were studied; *Lippia javanica, Ocimum lamiifolium, Croton macrostachyus, Azadiratchta indica, and Persea americana.* The *in-vivo* antidiabetic activity and safety of these extracts were screened in white male alloxan-induced diabetic albino mice. The aqueous plant extracts were administered orally and intraperitoneally. The safety of these plant extracts were studied by administering 450mg/kg, 670mg/kg and 1000mg/kg body weight orally and intraperitoneally daily for 28 days in mice. The mineral elements of the aqueous plant extracts were analyzed using atomic absorption spectrometry (AAS) and Total Reflection X-ray Fluorescence system (TRXF) while various phytochemicals present were qualitatively assessed using standard procedures. Results revealed antidiabetic activity of the extracts at varying doses of 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight. The extracts decreased the body weight gain and altered the organ to body weight percentage of the brain, kidney, liver, heart, testes and lungs for both intraperitoneal and oral routes. In both routes, administration of the same doses (450mg/kg, 670mg/kg and 1000mg/kg body weight) caused a change in levels of RBC, WBC, Hb, PCV, PLT, MPV, MCV, MCH, MCHC, neutrophils, lymphocytes, eosinophils, basophils, monocytes and biochemical parameters: AST, ALT, GGT, CK, α-AMYL, LDH, T-BIL, D-BIL, I-BIL, TG, TC, LDL-C, HDL-C, BUN, UA, Urea and Creatinine. The extracts contained tannins, flavonoids, saponins, sterols, anthraquinones and alkaloids. Elemental analysis confirmed the presence of Sodium, Chlorine, Potassium, Calcium, Titanium, Vanadium, Mercury, Chromium, Manganese, Iron, Copper, Zinc, Arsenic, Cadmium, Magnesium, Nickel and Lead at levels above or below the recommended daily allowance. In conclusion the results showed that the plant extracts were effective in reducing blood sugar levels the plants showed no toxicity and revealed the presence of vital phytochemicals and elements which posses' antidiabetic activities. The study therefore, confirmed the traditional use of these herbs and established their safety and efficacy data that can guide their proper use in the management of diabetes mellitus. Consideration should be made to carry out the same studies using higher animals. Besides, one can subject the plants to organic solvent extraction and compare activities of both aqueous and organic fractions. .

CHAPTER ONE

INTRODUCTION

1.1 Background information

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia due to absence of insulin or improper utilization of insulin by target cells (ADA, 2014). In the short term, uncontrolled diabetes is characterized by high blood glucose levels and defective metabolism of glucose, proteins and lipids (Lunze *et al.*, 2013). Patients with mild hyperglycemia do not experience any symptoms and, therefore, may be unaware that they have diabetes for many years. The presenting symptoms of hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision (Patel and Macerollo, 2010). Uncontrolled diabetes leads to hyperglycemia with ketoacidosis as well as the non-ketotic hyperosmolar syndrome (Chen *et al.,* 2010). The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, heart, nerves and blood vessels (Mukundi *et al*., 2015). Stunted growth, sexual dysfunction and susceptibility to opportunistic infections may also be associated with chronic hyperglycemia (Piero *et al*., 2015a).

The two main types of diabetes mellitus are: type I diabetes formerly called juvenile diabetes or insulin dependent diabetes mellitus (IDDM) and type II diabetes formerly called adult-onset diabetes or non-insulin dependent diabetes mellitus (NIDDM). Other forms of diabetes mellitus include secondary diabetes and gestational diabetes (Abdirahman *et al*., 2015). NDDM accounts for 90 percent of diabetic cases while IDDM accounts for only 5-10 percent of diabetics. Type II

is characterised by insulin resistance and β-cell dysfunction metabolic abnormalities (Tankoy *et al.,* 2008).

The number of people with diabetes mellitus is increasing due to population growth, aging, urbanization, and increase in prevalence of obesity and physical inactivity (Qamar *et al.,* 2011). It was estimated that the global prevalence of diabetes in the age groups between 20 to 70 years was 8.3% in 2013 and will be 10.1% by 2035 (IDF, 2006). Besides, the total number of adult with diabetes is projected to rise from 382 million in 2013 to 592 million in 2035. In sub-saharan Africa, diabetes mellitus prevalence was estimated to be 0.01% in 2000 and is projected to rise to 0.12% by the year 2025 (Shaw *et al.,* 2010). Estimates put the prevalence of diabetes mellitus in Kenya to be about 3.3%, the figure is projected to rise to 45 % by the year 2025 (Ministry of Health and Sanitation Kenya, 2010). In 2013, it was estimated that more than 5.1 million people had died from complications as a result of hyperglycemia. Approximately more than 80% of diabetic deaths occur in middle and low -income countries (Shaw *et al.,* 2010). Africa and Asia are identified as regions with greatest potential where diabetics could raise two to three folds above the present level (ADA, 2007).

As a result of the chronic nature of diabetes mellitus and the severity of its complications, diabetes is a costly disease, not only for affected individuals and their families, but also for the health systems (Piero *et al*., 2015a). According to the International Diabetes Federation (IDF), the cost for the treatment of diabetes globally in 2010 was about $ 376 billion (11.6% of total health spending). The IDF

predicts that these costs will increase by 2030 to $ 490 billion and it puts considerable strain on health systems (Whiting *et al.,* 2011).

The current therapies for diabetes mellitus are reported to be expensive, unavailable and have side effects (Nyamai *et al.,* 2016). For example, use of insulin and oral hypoglycemic agents are associated with drawbacks such as ineffectiveness on oral administration, short shelf life, requirement of constant refrigeration and in the event of excess dosage, fatal hypoglycemia ensues (Mukundi *et al.,* 2015). The use of oral hypoglycemic drugs like sulfonylureas and biguanides are also associated with tendency to gain weight (Tankoy *et al.,* 2008*).* Therefore, there is need to use effective, easily accessible and cheap means to manage diabetes mellitus (Murugi *et al.,* 2012).

Herbal medicines and traditional medical practitioners are receiving considerable attention from mainstream health officials, international medical research and training institutions (Nyamai *et al.,* 2015). Traditional medicine, in the estimate of the World Health Organization is used by up to 80% of the population of most developing countries especially in Africa (WHO, 2012). This is envisaged by strained economic situations of most African countries that drive African diabetics to seek cheaper treatment and management options (Piero *et al*., 2015b). Plant based herbal medicines are thought to be effective, safe and affordable (Surendran *et al*., 2011).

The hypoglycemic activity of some plants have been identified and experimentally demonstrated using *in-vivo* and *in-vitro* diabetic models and documented in several

studies. Some of these plants include: *Ocimum gratissimum* (Aguiyi *et al*., 2000; Egsie *et al*., 2006); *Zingeber officinale* (Ojewole, 2006). The chemical composition of herbal products and potency depends on the plant extract derivative, the age of the plant part used, season when harvested and the methods of processing (Whiting *et al.,* 2011). Among the traditionally used plants in the management of diabetes mellitus in Kijauri village Nyamira county Kenya are: *Lippia javanica, Ocimum lamiifolium, Croton macrostachyus, Azadiratchta indica* and *Persea americana.* The rationale for their utilization has rested largely on long-term clinical experience. However, there is limited scientific evidence regarding safety and efficacy to back up the continued therapeutic application of these herbal remedies. This therefore, makes it necessary to carry out a thorough scientific investigation to elucidate their hypoglycemic activities and safety profiles that will go a long way in validating their folkloric usage.

1.2 Problem statement

Diabetes mellitus and diabetes care are currently attracting considerable attention as it poses a big economic burden in both developed and developing countries with regards to health system costs, indirect costs arising from losses occasioned by patient disability and premature mortality, time spent by family members accompanying patients while seeking care, and intangible costs in terms of psychological pain to the family and loved ones (Murugi *et al.,* 2012). According to American Diabetes Association, the combined direct and indirect costs of diabetes in 1997 were estimated at US$98 billion in the United States of America. ADA, (2014a) reported that the total annual cost associated with diabetes in Latin America and the Caribbean to be US$65.216 billion and as high as US$50 million

in India for 200,000 type I diabetics. A study in India estimates that for a low-income Indian family with a diabetic adult, as much as 25% of the family income may be devoted to diabetes care. For families in USA with a child who has diabetes, the corresponding figure is 10% of the family income (WHO, 2010).

The mainstay of non-pharmacological treatment of diabetes mellitus is diet and physical activity (WHO, 2010). Other methods of treatment such as use of conventional drugs like exogenous insulin and oral hypoglycemic agents has been associated with drawbacks like their ineffectiveness, unavailability, short shelf life and costly nature; requirement of constant refrigeration and unwanted side effects such as fatal hypoglycemia and weight gain (Tankoy *et al.*, 2008*)*. The increased usage of some selected herbal medicines traditionally used in the management of diabetes mellitus in Nyamira county Kenya has not been accompanied by scientific evidence on their hypoglycemic activities and safety to support traditional medicine practitioners' claims. Therefore, such traditional medicines and practices can be harmful if therapy is of poor quality.

1.3 Justification of the study

In recent years, research interests have shifted to the search for alternative and complementary hypoglycemic agents, especially from plant sources. This is because plant based medicines are comparatively affordable, easily accessible, possess fewer side effects and are firmly embedded within wider belief systems of many people especially in underdeveloped and developing nations, thus according them greater acceptance. Besides, traditional medicines have been viewed by pharmaceutical industry as a source of 'qualified leads' in the production of

synthetic modern drugs. Therefore, in this study, *in vivo* evaluation of hypoglycemic activities and safety of some selected plants traditionally used in the management of diabetes mellitus was necessary to validate their herbal medicine utilization.

1.4 Hypotheses

i. Aqueous leaf extracts of the five selected medicinal plants do not possess hypoglycemic activities
ii. Intraperitoneal and oral administration of high doses of aqueous leaf extracts of the five selected medicinal plants have no *in vivo* toxicity in Swiss albino mice
iii. There are no mineral elements and phytochemical constituents associated with hypoglycemic activities in aqueous leaf extracts of five selected medicinal plants

1.5 Objectives

1.5.1 General objective

To determine *in vivo* hypoglycemic activities and safety of the five selected medicinal plants in Swiss albino mice

1.5.2 Specific objectives

i. To determine the *in vivo* hypoglycemic activity of aqueous leaf extracts of the five selected medicinal plants in Swiss albino mice
ii. To determine the *in vivo* safety of aqueous leaf extracts of the five selected medicinal plants in Swiss albino mice

iii. To determine the phytochemical and mineral elements composition of aqueous leaf extracts of the five selected medicinal plants.

CHAPTER TWO

LITERATURE REVIEW

2.1 Diabetes mellitus

Diabetes mellitus is a chronic metabolic disorder caused by an inherited and/or acquired impairements in production of insulin by the pancreas or by the ineffectiveness of the insulin produced (insulin resistance) (Ozougwu *et al.*, 2013). The condition is characterized by high blood glucose levels, which brings about various complications including damage to many of the body systems particularly blood vessels and nerves (Piero *et al.,* 2015b). Such damage of the body systems is caused by disturbances in the regulatory systems responsible for the metabolism, storage and utilization of carbohydrates, fats and proteins resulting from defects in insulin secretion, insulin action, or both (National Diabetes Data Group, 1979).

2.2 The prevalence and incidence of diabetes mellitus

The current research findings show that, the prevalence of diabetes mellitus is increasing globally (Wild *et al.,* 2004). It has now become an epidermic with a worldwide incidence of about 5%. This has been attributed to increase in prevalence of obesity, physical inactivity, population growth, aging, stress, and changes in food consumption (Qamar *et al.*, 2011).

According to WHO, the current world incidence of diabetes stands at 190 million people and it is projected that this figure will double by the year 2025. In developed countries most people with diabetes will be aged 65 years or more by year 2025 while in developing countries the affected age bracket will be between the ages 45-65 which are their most productive years (Mukundi *et al.*, 2015). In

Africa and Asia the prevalence of diabetes is expected to continue increasing as a result of changes in lifestyles and urbanization. Approximately, there are forty nine million diabetics in South-East Asia; 15 million people in India alone are diabetics and these accounts for almost a quarter of all patients in the region (Wild *et al.,* 2004).

It is estimated that 32.2 million patients in the European continent suffer from diabetes mellitus; out of which over a million of the cases come from United Kingdom alone. This accounts for over 2% of the UK population who suffer from the disease. Demographically, Latin America has an estimated 12.6 million diabetic patients while the Northern American continent has approximately 21.4 million. In the United States diabetes mellitus affects approximately 17 million adults and it is the third leading cause of death after heart disease and cancer. In Australia it is estimated that 700,000 people are diabetic and it is described as a disease of modern society. In the year 2000 there were approximately 7.5 million cases of diabetes in Africa and the figure is expected to rise to around 18.2 million by the year 2030. 80% of the cases in all African countries go undiagnosed as they struggle to take care of an ever increasing number of diabetic patients (Guariguata *et al.*, 2014).

In Kenya, diabetes was estimated to be 1.06% in 2000 and would possibly rise to 45% by the year 2025 (Ministry of Health and Sanitation Kenya, 2010). As a result of the chronic nature of diabetes mellitus and the severity of its complications, diabetes is a costly disease, not only for affected individuals and their families, but also for the health systems (Moura *et al*., 2013). Studies in India estimate that for a

low-income Indian family with a diabetic adult, as much as 25% of the family income may be devoted to diabetes care. For families in USA with a child who has diabetes, the corresponding figure is 10% (WHO, 2012). It is projected as the world`s main disabler and killer in the next 25 years (WHO, 2012).

2.3 Classification of diabetes mellitus

The type of diabetes mainly depend on the cause of diabetes mellitus, it can either be inherited or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. It results either from inadequate secretion of hormone insulin, an inadequate response of target cells to insulin, or a combination of these factors (Malviya *et al.,* 2010).

2.3.1 Type I diabetes mellitus

Type I diabetes mellitus is also known as insulin-dependent diabetes mellitus (IDDM) or juvenile onset diabetes (Malviya *et al.,* 2010). It occurs mainly due to β-cell destruction primarily as a result of cellular mediated autoimmune destruction of β-cells of the pancreas; inheritance (chromosomal and mitochondrial DNA mutation which results in abnormalities in the pancreas such as pancreatitis, pancreatomy, neoplasia, cystic fibrosis, fibrocalculous, pancreatopathy as well as diseases associated with pancreas such as acromegaly, Cushing's syndrome, glucagonoma, phaeochromocytoma, hyperthyroidism and aldosteroma); or it can be idiopathic (especially after congenital rubella and cytomegalovirus infection or administration/ exposure to drugs and chemicals such as pentamidine, nicotinic acid, glucorticoids, thyroid hormone, β-adrenargic antagonists, thiazides, and α–interferons. (Malviya *et al.,* 2010). In autoimmune type I diabetes, the rate of cell

destruction is quite variable, being rapid in some individuals and slow in others (Malviya *et al.,* 2010). It is prone to ketoacidosis (Gerich *et al.,* 2013).

Type I diabetes mellitus accounts for 5-10% globally of patients with diabetes (Harris *et al.,* 2012). In this form of diabetes, the rate of β-cell destruction is quite variable, being rapid mainly in infants and children and slow in adults. Children and adolescents may present with ketoacidosis as the first clinical manifestation of the IDDM, while others have modest fasting hyperglycemia that can rapidly change to severe hyperglycemia and/or ketoacidosis in the presence of infection or other stress (Stuart *et al*., 2008). Patients are completely reliant upon exogenous insulin to prevent ketosis and thereby preserve life (Harris *et al.,* 2012). The Caucasian population tends to present a higher risk for type 1 diabetes when compared to all other ethnic groups **(**Moura *et al.,* 2013).

2.3.2 Type II diabetes mellitus

Type II diabetes mellitus is also known as non-insulin-dependent diabetes mellitus (NIDDM) or adults onset diabetes whose onset is usually after 40 years of age and accounts for approximately 90-95% of the diabetes mellitus cases world-wide (Nwaobia *et al.,* 2012). It is mainly caused by insulin resistance and relative or absolute insulin deficiency (William *et al.*, 2008) and the risk of developing this form of diabetes increases with age, obesity, and physical inactivity (D, Souza *et al.*, 2005).

Most patients with NIDDM are obese or overweight, which causes some degree of insulin resistance (William *et al.,* 2008). This form of disease frequently goes

undiagnosed for many years because hyperglycemia develops gradually and earlier stages are often not severe enough for the patients to notice any of the classical symptoms of diabetes (Harris *et al.,* 2012).

Although, patients with NIDDM may have insulin levels that appear normal or elevated, higher blood glucose levels would be expected to result in even higher insulin values had their β-cells function been normal (William *et al.*, 2008). Thus, insulin secretion is defective in these patients and insufficient to compensate for insulin resistance.

2.3.3 Other forms of diabetes mellitus

Other forms of diabetes mellitus are; Gestational diabetes, diagnosed in pregnancy and affect 4% of all pregnant women (Harris *et al.,* 2012); the Brittle diabetes and maturity-onset diabetes of the young (MODY) associated with monogenic defects in β-cell function that is inherited in an autosomal dominant pattern, is characterized by onset of hyperglycemia at an early age, generally before age of 22 years and impaired insulin secretion with minimal or no defects in insulin action (Harris *et al.,* 2012).

2.4 Epidemiology of diabetes mellitus

Diabetes mellitus develops from a complex interaction between environmental and genetic factors. Its prevalence is characterized by a tremendous rise in the burden of non-communicable diseases. Heredity is a major factor in the development in diabetes mellitus (Gonzalez *et al.,* 2011). If both parents have type II diabetes, there is a chance that nearly all of their children will have diabetes. If both parents

have type I diabetes, fewer than 20 percent of their children will develop type I diabetes. If one of identical twins develops type II diabetes, chances are that nearly 100 percent the other twin will also develop it (Gonzalez *et al.,* 2011). In type I diabetes, however, only 40 to 50 percent of the second twins will develop the disease, indicating that while inheritance is important, environmental factors (for example, food, stress, viral infection, among others) are also involved in the development of type I diabetes (Murugi *et al*., 2012).

Increase in obesity rates is to blame for much of the increase especially of type 2 DM. In the U.S.A two-thirds of the U.S adults are reported to be overweight or obese, as a result predictions show that one in three US citizens born in 2000 will develop diabetes mellitus (Llorente and Malphurs, 2007). Habits characterized by low energy expenditure daily and by excessive ingestion of foods rich in carbohydrates and lipids, result in positive energy balance leading to, increase of the body mass index (BMI) and prevalence of obesity, in developed as well as developing countries (Carvalho *et al*., 2012).

2.5 Signs and symptoms of diabetes mellitus

The presenting symptoms of hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision (Stuart *et al*., 2008). Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia. Life threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or the non-ketotic hyperosmolar syndrome (Stuart *et al*., 2008). Long term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy

with risk of foot ulcers, amputations, Charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction (Alberti *et al.*, 1998).

2.6 Predisposing factors of diabetes mellitus

Research has established that the following are the major predisposing factors for diabetes mellitus**:**

i. Advanced age; older people are more predisposed to obesity than younger people (Gonzalez *et al.,* 2011).

ii. Family history; people who have a strong family history of diabetes such as parent or sibling are at a high risk of developing diabetes (Gonzalez *et al.,* 2011).

iii. Excessive body weight; adults and children who are overweight and obese are physically inactive, weight gain may result in insulin resistance through such mechanisms as placing a greater demand on the pancreas to produce insulin, in such a situation the body is unable to use the insulin it produces effectively, physical inactivity is both a cause and consequence of weight gain (Gonzalez *et al.,* 2011). Moreover, it has been found that there is a strong association between obesity and chronic diseases such as diabetes, cardiovascular diseases, hypertension, osteoarthritis, some cancers and inflammation based pathologies which suggest that the obese are likely to have a disproportionate use of the health care system (Williams *et al*., 1982).

iv. Ethnicity; it has been reported that certain ethnic groups have higher rates of type 2 diabetes mellitus especially those of Asian, African and indigenous peoples of America and Australia (Beaglehole, 2003).

v. Stress, unhealthy diet and women who developed diabetes during pregnancy (gestational diabetes) are at a greater risk of type 2 diabetes later in life (Wild *et al.,* 2004).

vi. There is evidence associating use of certain substances with diabetes, for example alcohol, heavy alcohol drinking may lead to chronic pancreatitis or cirrhosis of the liver, which brings about direct damage to the pancreatic β-cells (Stuart *et al*., 2008). Smoking raises systolic pressure and makes hypertension difficult to control; hypertension is a common co-morbidity of diabetes. Drugs like marijuana leads to increased appetite for food especially sugars and may impair judgement and reasoning skills, resulting in noncompliance or errors in judgement (Stuart *et al*., 2008).

2.7 Diagnosis of diabetes mellitus

Diabetes Mellitus is a disease, which by virtue of its complications may affect all organ systems in the body. Prevention, timely diagnosis, and treatment are important in patients with diabetes mellitus. Many of the complications associated with diabetes, such as nephropathy, retinopathy, neuropathy, cardiovascular disease, stroke, and death, can be delayed or prevented with appropriate treatment of elevated blood pressure, lipids, and blood glucose (Alberti *et al*., 1998).

The body usually is able to keep glucose concentrations stable. The normal fasting blood sugar is usually between 3.5-6.7mmol/l. After a meal it would rarely exceed

8mmol/l. Normally there is no glucose in urine since the normal threshold above which glucose would appear in the urine would be 10mmol/l. Below a concentration of 10mmol/l the kidneys reabsorb glucose back into the blood stream and so glucose does not appear in the urine unless the blood concentration of glucose is high (TA *et al*., 2014). Some of the diagnostic tests for diabetes mellitus include:

2.7.1 Oral glucose tolerance test (OGTT)

The oral glucose tolerance test evaluates clearance from the circulation after glucose loading under defined and controlled conditions (Mari *et al*., 2001). The patient need have been fasting 8-14 hours prior to sampling. Before oral administration of the glucose solution within 5 minutes a zero time (baseline) blood sample is drawn. Blood is then drawn at 2-hour intervals for measurement of glucose (blood sugar), and sometimes insulin levels. The zero and 2-hour samples may be the only ones collected. In a non-diabetic, the blood glucose level increases immediately after a high sugar carbohydrate drink and then decreases gradually since high blood sugar levels stimulates insulin release from the beta cells that enhances upatake of glucose by peripheral tissues from the blood stream resulting to its low levels (Test, 2002). In a diabetic, the glucose in the blood continues to go up and stays high after drinking the sweetened liquid. Therefore, a plasma glucose level of 200 mg/dL or higher at two hours after drinking the sweetened syrup and at one other point during the two hour test period confirms the diagnosis of diabetes mellitus (TA, 2014).

During the test, the patient must be ambulatory, since inactivity decreases glucose tolerance. The test can also be affected by illness, abnormalities of such hormones as thyroxin, growth hormone, cortisol, and catecholamines, drugs and medications such as oral contraceptives, salicylates, nicotinic acid, diuretics and hypoglycaemic agents and testing time. The glucose load should consist of glucose only (ADA, 2014b).

2.7.2 Postprandial plasma glucose test

Diabetes mellitus is more readily detected when carbohydrate metabolic capacity is tested (ADA, 2013). This can be done by stressing the system with a defined glucose load. Measurement of the rate that the glucose load is cleared from the blood, as compared to the rate of glucose clearance in healthy persons, detects impairment in glucose metabolism (ADA, 2013).

A meal high in carbohydrates is used as the carbohydrate load, although a 75g glucose drink is usually preferred over a meal. Blood is drawn at 2 hours after ingestion of the meal or glucose drink. Glucose levels above 1400mg/L are abnormal; levels of 1200 to 1400 mg/L are ambiguous; and levels below 1200mg/L are normal (Ngugi *et al*., 2012a). Though widely used for detection of diabetes mellitus, this test method is highly inaccurate due to several variables that are difficult to control or adjust for these variables such as age, weight, previous diet, activity, illness, medications, time of the day that the test is conducted and actual size of the glucose dose. When a meal is used as the load, the effective glucose load depends on the digestion of disaccharides and polysaccharides and their subsequent absorption from the intestinal tract (Ngugi *et al*., 2012a).

2.7.3 Urine tests

Urine tests are undertaken to analyze ketones bodies, glucose and proteins in the urine (Goldstein *et al*., 2004). The colorimetric reaction that occurs between ketones and nitroprusside (Sodium nitroferricyanide) is the method used for the rapid semiquantitive measurements of ketones (Goldstein *et al*., 2004). Diabetics who are under control exhibit wide variation in their plasma glucose concentrations. Plasma glucose levels in controlled diabetics range during a typical 24- hour period from as low as 250mg/L to as high as 3250mg/L. These variations are wider than those of nondiabetics (Saudek *et al*., 2006).

2.7.4 Intravenous glucose tolerance test (IGTT)

The intravenous glucose tolerance test is used for persons with malabsorptive disorders or previous gastric or intestinal surgery (Ngugi *et al*., 2012a). Glucose is administered intravenously over 30 minutes, using a 20% solution. A glucose load of 0.5g/kg of body weight is used. Nondiabetics respond with a plasma glucose level of 2000 to 2500mg/L. Discontinuation of the glucose loading leads to a decrease in plasma levels with fasting levels reached at about 90 minutes. Diabetics demonstrate plasma glucose level above 2500mg/L during administration of the load. On discontinuation of the loading, plasma glucose levels of diabetics also return to fasting levels at about 90 minutes (Mari, *et al*., 2001). An alternative procedure called the Soskin method uses 50% glucose delivered intravenously within 3 to 5 minutes. The glucose load used is 0.3 g/kg of body weight. Nondiabetics re-establish fasting levels in less than 60 minutes after discontinuing the glucose infusion. In diabetics fasting levels are established significantly later than 60 minutes (Mari *et al*., 2001).

2.7.5 O'Sullivan test

This test is used to detect gestational diabetes. A 50 g load of glucose is given to a fasting patient. Blood is drawn at one hour. Gestational diabetes is suggested by plasma levels above 1500mg/L (O'sullivan and Mahan, 1964).

2.7.6 Glycated hemoglobin

In diabetes mellitus, a minor hemoglobin derivative called HbA1c is produced by glycosylation. Since this reaction is spontaneous and erythrocytes are completely permeable to glucose, the quantity of HbA1c formed is directly proportional to the average plasma glucose concentration that the erythrocytes are exposed to during their 120-day life span (4 to 6 weeks before sampling) (Selvin, *et al*., 2010). For normoglycemic persons, HbA1c constitutes 4% to 5% of total hemoglobin, whereas in diabetics, HbA1c levels are significantly elevated. The elevations are directly proportional to the long-term degree of hyperglycemia. Glycated hemoglobins are the most useful for monitoring of diabetes mellitus. However, they are not sufficiently sensitive to effectively detect borderline cases of diabetes mellitus. Serum albumin is also glycosylated to a degree proportional to plasma glucose levels. The short half-life for albumin of 15 days makes it a good monitor of short-term blood plasma glucose levels (Selvin, *et al*., 2010).

2.7.7 Plasma insulin test

Fasting plasma insulin levels in type I diabetics are low. Those of type II diabetics are low only when fasting plasma glucose levels exceed 2500mg/L. Otherwise, they are normal. A glucose challenge separates type I diabetics from type II diabetics. Glucose loading elicits no significant insulin response for type I

diabetics and a delayed, exaggerated response in type II diabetics (Weyer *et al*., 2001). Diabetics can monitor their own blood glucose levels with home blood glucose monitoring kits. A small needle or lancet is used to prick the finger and a drop of blood is collected and analyzed by a monitoring device. The correct use of such a device minimizes the wide variations of blood glucose experienced by diabetics and, as a result, the hypoglycemic events and even the longterm complications of diabetes mellitus. Blood glucose levels maybe tested several times during the day and use the information obtained to adjust their diet or doses of insulin (Weyer *et al*., 2001).

In a consensus statement on blood glucose monitoring, many insulin-treated populations have been recommended for self-monitoring programmes. These include pregnant women, patients with unstable diabetes, patients with histories of severe ketosis or hypoglycemia especially those who do not demonstrate warning symptoms of hypoglycemia, patients receiving intensive insulin therapy and patients with abnormal renal thresholds for glucose (Weyer *et al*., 2001).

2.8 Complications of diabetes mellitus

Diabetes mellitus is a very expensive disease whose complications reduce both life expectancy and quality of life. It therefore, has profound implications in terms of long-term microvascular and macrovascular complications and their associated cost. The in-depth discussion of the complications of diabetes mellitus is as follows:

2.8.1 Diabetic neuropathy

It is a condition that is associated with nerve damage**,** diabetic neuropathy can be classified as peripheral, autonomic, proximal or focal, depending on the affected body part, focal neuropathy is the less prevalent and is generally acute and self-limiting (Jabbour *et al.,* 2008; Moura *et al.,* 2013). Peripheral neuropathy is the most common manifestation with chronic sensorimotor symptoms and signs, the onset of which is usually insidious, it may be asymptomatic in about 50% of patients, 10% to 20% of patients may have sensory symptoms necessitating treatment (Smith *et al.,* 2012). Diabetic neuropathy is more frequent in older people, on average neuropathy symptoms begin to appear within 10–20 years of the diagnosis of diabetes, and approximately 50% of diabetic patients will develop nerve damage (Moura *et al.,* 2013).

Diabetic neuropathy is the most common cause of foot ulcers in diabetic population, and is associated with increasing the likelihood of amputations especially in western countries (Llorente and Malphurs, 2007). Diabetic neuropathy develops as a result of hyperglycemia which inhibits the normal uptake of myoinositol, leading to a decrease in the myoinositol within the nerve. This action prolongs nerve conduction, causing nerve dysfunction. Long-term hyperglycemia also activates the polyol pathways (Xu *et al.,* 2012). The excess glucose is converted to sorbitol and fructose, causing the accumulation of sorbitol and fructose in nerve cell. Their accumulation leads to intracellular osmotic stress (Xu *et al.,* 2012).

2.8.2 Diabetic nephropathy

Diabetic nephropathy occurs due to damage of the glomeruli and its associated capillaries in the kidney causing protenuria. 25% to 30% of individuals treated for end-stage renal failure are diabetics with type 2 form of diabetes (Votey and Peters, 2005). All diabetic patients have the potential for renal impairment unless otherwise proven. It is advisable to effect referral of patients with diabetes who are hypertensive for long-term blood pressure management since chronic blood pressure elevation contributes to the decline in renal failure (Votey and Peters, 2005).

2.8.3 Angiopathy

Chronic diabetes causes a disease of the blood vessels (arteries, veins, and capillaries) known as angiopathy (Krolewski *et al.,* 1987). The two types of angiopathies are macroangiopathy and microangiopathy (Krolewski *et al.,* 1987). Macroangiopathy is characterized by build up of fat and blood clots in the large blood vessels that stick to the vessel walls blocking the flow of blood. In microangiopathy, the smaller blood vessels walls become so thick and weak causing bleeding, leaking of proteins, and slowing the flow of blood throughout the body. These therefore, deprives nourishment to cells especially those in the center of the eye which eventually get atrophied or necrotic. Studies have demonstrated that angiopathy increases the risk of coronary heart disease and stroke and leads to nephropathy and retinopathy (Krolewski *et al.,* 1987).

2.8.4 Diabetic cataracts

Chronic hyperglycemia is commonly associated with cataracts. The epithelial cells of the lens do not require presence of insulin to metabolize glucose since they contain the enzyme aldose reductase, which converts glucose into sorbitol (Gabbay, 1975). Since sorbitol is difficult to traverse the plasma membranes it is retained inside the cells. Sorbitol dehydrogenase then converts sorbitol into fructose. This raises the intracellular osmolality leading to increased water uptake and swelling of the lens (Gabbay, 1975).

2.8.5 Retinopathy

Diabetes mellitus also cause damage to the tiny blood vessels in the retina resulting in diabetic retinopathy (Wilkinson *et al.,* 2003). Most people usually do not notice any changes in their vision immediately. Others often develop a condition known as macular edema. Macular edema occurs when the damaged blood vessels leak fluid and lipids onto the macula, the part of the retina that lets us see detail. The fluid therefore, makes the macula swell, blurring vision. If the disease is not treated early it progresses into its advanced or proliferative stage. The resultant fragile, new, blood vessel grows along the retina in a clear, gel-like vitreous humour that fills the inside of the eye causing cloud vision, bleeding of blood vessels and eventually destroys the retina (Wilkinson *et al.,* 2003).

2.8.6 Diabetic foot

Foot infection involving the skin and soft tissues is a common complication in diabetic individuals and represents a major cause of morbidity and mortality and is a major reason for lower limb amputation (Crouzet *et al*., 2011). The foot of

diabetic patients becomes susceptible to both ischemic and neuropathic ulceration. Neuropathic ulceration is the consequence of traumatic damage to the skin in the presence of sensory loss, especially when accompanied by mechanical derangement of the foot (Scobie, 2002).

The estimated annual total cost of diabetes related to foot complications in the UK is approximately $252 million inclusive of $8459 for every single amputation (Paton, 2011). Patients with diabetes have a 12-25% life time risk of developing foot ulcers, major amputations are done when the ulcerated foot either threatens patient survival or when reasonable function can no longer be expected (Leung, 2007).

2.8.7 Sexual dysfunction

Both physiological and psychological factors can contribute to sexual dysfunction among patients with diabetes mellitus. Normal human sexual activity is composed of four stages desire, arousal, orgasm and resolution (Llorente and Malphurs, 2007). Sexual dysfunction is prevalent among men with diabetes occuring two to four times more often than in persons without diabetes. Whereas sexual dysfunction most commonly develops after the age of 60 years, it tends to occur 5-10 years earlier among men with diabetes (Llorente and Malphurs, 2007). Diabetes may affect arousal due to decreased genital sensation and lubrication. Women with type two diabetes mellitus are also predisposed to vaginal dryness and infections which can lead to dyspareunia (Llorente and Malphurs, 2007).

2.8.8 Hyperlipoproteinemia

Diabetics are also characterized by abnormal circulating lipids. There is usually increased Very Low Density Lipoprotein (VLDL), which is manifested by elevation of plasma triacylglycerol and cholesterol but the former predominates (Gotoda *et al.,* 2012). Hyperglcemia is associated with insulin deficiency and enhanced lipolysis. Therefore, glucose and free fatty acids are transported to the liver, where they are utilized for VLDL synthesis. Elevated levels of Very Low-Density Lipoproteins (VLDL) are a characteristic of type 2 diabetics and this complication predisposes individuals to atherosclerosis and myocardial infarction. However, High-Density Lipoproteins (HDL) are lower in diabetics (Gotoda *et al.*, 2012).

2.8.9 Diabetic ketoacidosis

High blood sugar levels or hyperglycemia causes Diabetic ketoacidosis (DKA). Deficiency of the hormone insulin is a key component of DKA and therefore, without insulin, glucose cannot be transported out of the bloodstream into the cells (Wolfsdorf *et al.,* 2006). Unavailable glucose within the cells triggers synthesis of glucose from non-carbohydrate sources such as proteins and lipids (Wolfsdorf *et al.,* 2006). Metabolism of proteins and lipids results in ketoacids leading to elevated hydrogen ions. Increased levels of ketone bodies and high blood sugar significantly increase body osmolality altering the pH of blood. Eventually glycosuria sets in and results to dehydraration which worsens the increased osmolality forcing water out of cells and into the bloodstream in order to keep vital organs perfused. If untreated, the vicious cycle will lead to coma and death (Jiang and Zhang, 2003; Wolfsdorf *et al.,* 2006).)

2.8.10 Effects of diabetes on the foetus

Spontaneous abortion, birth defects, and macrosomal are the adverse complications the fetus of a pregnant diabetic is at risk of developing if greater glycemic control is not enforced, especially during the early weeks of pregnancy (Hay, 2012). Moreover, the risk for these complications is directly related to the degree of maternal hyperglycemia (Hay, 2012).

2.9 Management of diabetes mellitus

2.9.1 Oral antidiabetic drugs

The overall focus for management of diabetes mellitus is to minimize chronic diabetic complications, avoid acute problems of hyperglycemia or hypoglycemia, blindness, heart disease and limb amputation (Tuomilehto *et al.,* 2001). Patients with type I diabetes mellitus require direct injection of insulin as their bodies cannot produce enough (or even any) insulin. For type II diabetics, diabetic management consists of a combination of diet, exercise, and weight loss, in any achievable combination depending on the patient. Therefore, type II diabetic patients who thereafter have poor diabetic control are typically placed on oral hypoglycemic (Tuomilehto *et al.,* 2001). However some type II diabetics fail to respond to these and must proceed to insulin to compensate for relative insulin deficiency. The oral glucose-lowering drugs are used for management of type II diabetes mellitus. Conventionally, oral therapy is indicated in any type II diabetic in whom diet and exercise fail to achieve acceptable glycaemic control. However, although initial responses may be good, oral hypoglycaemic agents lose their effectiveness in a good number of diabetics (Tuomilehto *et al.,* 2001). Some of oral hypoglycemic agents include:

2.9.1.1 Sulfonylureas

Sulfonylureas have played an important role in hyperglycemia management because of their potency, fast action, and relative low cost. Research have confirmed that first-line therapy with sulfonylureas in newly diagnosed type II diabetes mellitus is a safe and effective treatment for glucose control (Man-Wo Tsang, 2012). They stimulate insulin secretion from the β-cell. They appear to sensitize the β-cell to various other insulin secretagogues, such as glucose. An improvement in insulin resistance may also be observed with sulfonylureas (Patil *et al.,* 2011).

The first-generation agents (acetohexamide, chlorpropamide, tolazamide, and tolbutamide) have a lower binding affinity to the receptor on the β-cells, so they must be given in higher doses than the second generation agents, (glimepiride, glipizide, gliclazide, and glyburide) which have a higher binding affinity. Among the second generation agents, there are difference in their differential binding specificity to beta-cell SUR-1 and SUR-2 in cardiac muscle (Man-Wo Tsang, 2012). The commonest adverse effects of sulfonylureas are hypoglycemia, which can be severe and prolonged. Allergic skin rashes can occur as well as bone marrow damage although very rare (Patil *et al.,* 2011). Sulfonylureas have a tendency to produce weight gain, although intervention that improves diabetic control in a patient following an isocaloric diet would be expected to result in such an effect (Scobie *et al.,* 2002).

2.9.1.2 Biguanides

These compounds increases insulin sensitivity by reducing hepatic gluconeogenesis (primary effect), reducing plasma triglycerides and LDL-Cholesterol levels, increasing peripheral insulin sensitivity (secondary effect) and increasing skeletal muscle glucose uptake (Al-Arouj *et al.,* 2010). They do not increase insulin levels or cause weight gain. Taken alone, they do not cause hypoglycemia. An example of biguanides is metformin (phenformin) (Milleri *et al.,* 2013).

The major target of metformin is the enzyme AMP-activated protein kinase (AMP-kinase). Activation of AMP-kinase by metformin results in decrease of hepatic glucose production and increase glucose transport in skeletal muscle (Man-Wo Tsang, 2012). It is used as monotherapy or in combination with sulfonylureas for management of type II diabetes mellitus. The use of metformin is contraindicated in patients with renal insufficiency (abnormal creatinine clearance) or in acute or chronic metabolic acidosis (Milleri *et al.,* 2013). However, side effects of metformin are weakness, fatigue, and shortness of breath, nausea, dizziness, lactic acidosis, and kidney toxicity (Leiter *et al.,* 2015).

2.9.1.3 α-Glucosidase inhibitors (α-Gis)

Alpha-glucosidase inhibitors, inclde Acarbose (Precose) and Miglitol (Glyset), which are indicated as monotherapy or in combination with sulfonylureas for management of type II diabetes (Rabasa-Lhoret and Chiasson, 2004). They reduce post-prandial glucose levels by inhibiting the breakdown of complex carbohydrates and delay the absorption of monosaccharides from the gastrointestinal tract. They

inhibit the action of α-glycosidase, the enzyme responsible for digesting carbohydrates, in the intestine, thus delaying and attenuating postprandial blood glucose peaks. Undigested sugar is delivered to the colon, where it is converted into short-chain fatty acids, methane, carbon dioxide, and (Rabasa-Lhoret and Chiasson, 2004).

2.9.1.4 Meglitinides

These are short-acting insulin secretagogues that act on ATP dependent potassium channels in pancreatic β-cells, allowing opening of calcium channels and increased insulin release (Rudovich *et al.,* 2010). They include repaglinide. Repaglinide has a rapid onset of action and is taken with meals two to four times daily. It is a suitable option for patients with severe sulfur allergy who are not candidates for sulphonylurea therapy. The drug is used as monotherapy or in combination with metformin. It is titrated cautiously in elderly patients and in those with renal or hepatic dysfunction. The side effects of repaglinide include weight gain, gastrointestinal disturbances, and hypoglycemia (Rudovich *et al.,* 2010).

2.9.1.5 Thiazolidinediones

Thiazolidinediones are "insulin sensitizers" that promote skeletal muscle glucose uptake (Yki-Järvinen, 2004). They improve insulin sensitivity in muscles. In the liver they decrease plasma triglyceride levels, but such decreases are associated with weight gain and an increase in LDL-cholesterol levels (Yki-Järvinen, 2004). These agents work by binding to peroxisome proliferator-activator receptor-γ (PPAR-γ), which is primarily located on adipocytes (Miyazaki *et al.,* 2002). Thiazolidinedione therapy is associated with a reduction in circulating plasma free

fatty acid (FFA) levels and FFA turnover, a decrease in hepatic fat content, and an improvement in peripheral insulin sensitivity In type II diabetic patients (Parulkar *et al.,* 2001). They include such drugs as troglitazone, rosiglitazone and pioglitazone. Troglitazone (Rezulin) reduces insulin resistance (Miyazaki *et al.,* 2002). It is beneficial in patients requiring large daily amounts of insulin (more than 30 units per day) whose diabetes is still uncontrolled. Rosiglitazone (Avandia) is an insulin sensitizer that stimulates glucose uptake by skeletal muscle and adipose tissue. Pioglitazone (Actos) improves target cell response to insulin without increasing insulin secretion from pancreas. Actos decreases hepatic glucose output and increases insulin-dependent glucose use in skeletal muscle and liver.

2.9.1.6 Insulin therapy

When glycemic control is suboptimal at maximal doses of oral medications insulin is added as an oral agent to compensate the need (Van den Berghe *et al.,* 2006). Type I diabetics require lifelong treatment with insulin to promote glucose utilization. Insulin therapy is initiated in patients with newly diagnosed type II diabetes. Hypoglycemia and weight gain are common side effects associated with insulin therapy. Continued insulin administration also carries an increased risk of atherogenesis (Pittas *et al.,* 2004). Short-, intermediate-, and long-acting insulin preparations are used. Pork, beef, and beef-pork insulins were used previously, but at present, recombinant human insulin is used (Van den Berghe *et al.,* 2006).

2.9.2 Exercise and diabetes mellitus

Exercise training raises high density lipoprotein cholesterol, lowers blood pressure, and leads to a 20- 40% increase in insulin sensitivity by enhancing insulin action in skeletal muscles (Jabbour *et al.,* 2008). All diabetic patients should be encouraged to engage in 30 minutes of modest aerobic exercise 3-4 times per week. The intensity should be gauged to produce an increase in pulse rate to 60-70% of maximum which can be calculated as 220-minus age (Jabbour *et al.,* 2008*).* However exercise may not be ideal for long term care because it is associated with potential risks such as cardiac ischemia musculoskeletal injuries, and hypoglycemia in patients treated with insulin or secretagogues (Fieldman, 2009).

2.9.3 Nutritional management of diabetes mellitus

Nutrition is often said to be the cornerstone of diabetes care (Meltzer *et al.,* 1997). For many individuals with diabetes, the most challenging part of the treatment plan is determining what to eat. It is the position of the American Diabetes Association (ADA) that there is not a "one-size-fits-all" eating pattern for individuals with diabetes (ADA, 2015). The nutritional management of diabetes can affect long term health and quality of life. The goal for nutritional management is optimal metabolic control through a balance between food intake, physical activity, and if necessary, medication to avoid complications. In type 2 diabetes, nutritional goals aim for improved glycemic and lipid levels and weight loss when required (Inzucchi *et al.,* 2015). Because diabetes is a state of increased oxidative stress, antioxidant vitamins are prescribed to people with diabetes. Examples include:

i. **Vitamins B_6:** Vitamin B_6 supplementation offers significant protection against the development of diabetic neuropathy as diabetes patients with

neuropathy are deficient in vitamin B_6. It also inhibits glycosylation of proteins. Individuals with long-standing diabetes or who are developing signs of peripheral nerve abnormalities are supplemented with vitamin B_6. The neuropathy of a vitamin B_6 deficiency is indistinguishable from diabetic neuropathy. (Head, 2006).

ii. **Vitamin C:** Vitamin C reduces glycosylation and offers antioxidant protective mechanism, which is beneficial to diabetics (Sheikh-Ali *et al.*, 2011).

iii. **Vitamin E:** Vitamin E provides lipophilic antioxidant activity, with possible influences on protein glycation, lipid oxidation, and insulin sensitivity and secretion. It also affects non-oxidative glucose metabolism (O'Connell, 2001).

iv. **Vitamin B12:** Vitamin B_{12} supplementation has been used with some success in treating diabetic neuropathy. Although it is not clear if this is due to the correction of a deficiency state or normalization of the deranged vitamin B_{12} metabolism seen in diabetic patients. A deficiency in vitamin B_{12} is characterized by numbness of the feet, pins and needles sensations, or a burning feeling. (Franz *et al.*, 2002).

v. **Biotin:**. Biotin supplementation enhances insulin sensitivity and increases the activity of the enzyme glucokinase, the enzyme responsible for the first step in the utilization of glucose by the liver. Glucokinase concentrations in diabetes patients are very low. It also improves nerve function (Reddi *et al.*, 1988).

vi. **α -lipoic acid:** α-lipoic acid is a potent lipophilic antioxidant which act as a cofactor in many multienzyme complexes such as pyruvate dehydrogenase

multienzyme complex. It also plays a role in glucose oxidation. *In vitro* α-lipoic acid enhances glucose uptake in muscle and prevents glucose-induced protein modifications (Ametov *et al.,* 2003).

2.9.4 Mineral elements and antidiabetic activity

Mineral elements have multiple roles within the body. Macro-elements work together with vitamins and initiate hormone production as well as speeding up the metabolic processes. Trace elements participate in tissue and cellular and subcellular functions; these include immune regulation by humoral and cellular mechanisms, nerve conductions, muscle contractions, membrane potential regulations, mitochondrial activity, and enzyme reactions. Trace elements interact with vitamins and macro-elements to enhance their effects on the body (Siddiqui *et al.,* 2014). They are accepted as essential for human health and have diverse metabolic characteristics and functions as described below:

i. **Magnesium:** Magnesium is an essential ion involved in multiple levels in insulin's secretion, its binding and its activity; cofactor in various enzyme pathways involved in glucose oxidation, and it also modulates glucose transport across cell membranes (Salmonowicz *et al.,* 2014). The normal level of serum magnesium ranges between 1.8 and 3.0 mg/dL (0.8–1.2 mmol/L) (Salmonowicz *et al.,* 2014). Magnesium increases insulin secretion and/or improves insulin sensitivity and peripheral glucose uptake. It has no effect on hepatic glucose output and non-oxidative glucose disposal (Salmonowicz *et al.,* 2014). Deficiency of magnesium potentially causes states of insulin resistance thus it is significantly more common in type 2 diabetics than in the general population especially those with

glycosuria, ketoacidosis, and excess urinary magnesium losses (Lee, 2010). Magnesium deficiency is associated also with complications of diabetes, retinopathy in particular. One study observed that the patients with the most severe retinopathy had also the lowest levels of magnesium. Lowering of serum magnesium has been shown in patients of long term treatment with insulin and those recovering from ketoacidosis (Rajendra *et al.,* 2007).

ii. **Zinc:** Zinc plays an important role in glucose metabolism. It helps in the utilization of glucose by muscle and fat cells. It is required as a cofactor for the function of intracellular enzymes that may be involved in protein, lipid, and glucose metabolism. Zinc may be involved in the regulation of insulin receptor-initiated signal transudation mechanism and insulin receptor synthesis (Rajendra *et al.,* 2007). Zinc forms a structural part of key antioxidant enzymes such as superoxide dismutase, and zinc deficiency impairs their synthesis, leading to increased oxidative stress. Zinc has a biphasic effect in that it is required for insulin storage and cellular binding, although high concentrations can lead to a reduction in insulin release (Rajendra *et al.,* 2007).

iii. **Potassium:** Potassium supplementation yields improved insulin sensitivity, responsiveness and secretion; insulin administration induces a loss of potassium; and a high potassium intake reduces the risk of heart disease, atherosclerosis, and cancer (Rajendra *et al.,* 2007). Normal potassium concentration is nesccessary for optimal insulin secretion. Potasium deficiency results more often from excessive losses than from deficiency intakes. Deficiencies arise in abnormal conditions such as diabetic acidosis.

Potassium depletion can result in reduced glucose tolerance (Rajendra *et al.,* 2007).

iv. **Vanadium:** Vanadium affects various aspects of carbohydrate metabolism including glucose transport, glycolysis, glucose oxidation, and glycogen synthesis (Siddiqui *et al.,* 2014). Vanadyl sulfate at a dose of 100 mg/day improves insulin sensitivity (Siddiqui *et al.,* 2014). It's mechanism of action in glycemic control is thought to be primarily insulin-mimetic with up regulation of insulin receptors. In animal models, it facilitates glucose uptake and metabolism and enhances insulin sensitivity (Siddiqui *et al.,* 2014). Clinically, it enhances glucose oxidation and glycogen synthesis, and it modulates hepatic glucose output. Gastrointestinal discomfort, including diarrhea, nausea, and flatulence, are the side effects of administration of vanadium salts to patients. Organically chelated compounds, however, are thought to cause less gastrointestinal irritation than vanadium salts (Siddiqui *et al.,* 2014).

v. **Iron:** Iron is both an essential nutrient and a potential toxicant to cells. The sufficient supply of iron is essential for the functioning of many biochemical processes, including electron transfer reactions, gene regulation, binding and transport of oxygen, regulation of cell growth, and differentiation, and is also involved in the proper function of immune system (Siddiqui *et al.,* 2014). Impaired glucose metabolism and diabetes mellitus are common clinical manifestations of iron overload in patients with hemochromatosis. Recently, moderately elevated iron stores below the levels commonly associated with hemochromatosis have also been implicated in the etiology of diabetes. Ferritin is a ubiquitous intracellular

protein which serves as a marker for tissue iron stores. Levels of plasma ferritin are elevated in persons with prevalent diabetes as compared with non-diabetic controls (Siddiqui *et al.,* 2014).

vi. **Manganese:** Manganese is essential for human health. It functions as a key constituent of metallo-enzymes activator in cellular biochemical reactions (Siddiqui *et al.,* 2014). Such reactions include making and activating manganese superoxide dismutase (MnSOD) (an antioxidant enzyme) that helps protect the cell membranes and tissues from degeneration and disruption, helping the body to catabolize carbohydrates, lipids and proteins, and assisting in energy production. Manganese deficiency causes impaired glucose tolerance, impaired growth, impaired reproductive function, skeletal abnormalities, and altered carbohydrate and lipid metabolism (Lee, 2010). Manganese supplements have reversed the impaired glucose utilization induced by manganese deficiency in guinea pigs (Rajendra *et al.,* 2007). Manganese may act like insulin in increasing the transport of glucose into adipose tissue either by enhancing an existing low level of insulin. It is a constituent of some enzymes such as pyruvate carboxylase and arginase and an activator of different enzymes such as phosphoenolpyruvate carboxykinase (PEPCK) and glutamine synthetase (Siddiqui *et al.*, 2014).

vii. **Chromium:** Chromium is required for normal carbohydrate metabolism and as a critical cofactor for insulin action (Siddiqui *et al.,* 2014). It is a part of glucose tolerance factor (GTF), a biologically active substance manufactured in the body that regulates glucose biotransformation and increases the number of insulin receptors, enhances receptor binding, and

potentiates insulin action (Siddiqui *et al.,* 2014). Experimentally, chromium deficiency is associated with impaired glucose tolerance, which is improved with supplementation (Lee, 2010). Chromium administration decreases fasting and postprandial glucose and decreases fatigue, excessive thirst, and frequent urination (Siddiqui *et al.,* 2014).

viii. **Copper:** Copper is considered as both a powerful enzyme catalyst and a dangerous reactant that generates hydroxyl radical (Siddiqui *et al.,* 2014). A deficiency of copper results in glucose intolerance, decreased insulin response, and increased glucose response. It is associated with hypercholesterolemia and atherosclerosis (Siddiqui *et al.,* 2014). Copper possesses an insulin-like activity and promotes lipogenesis. Copper is required for absorption and transport of iron and it plays a key role in haemoglobin synthesis. (Rajendra *et al.,* 2007).

ix. **Calcium:** Calcium and cyclic AMP are important in the stimulation of insulin release. The increase in the concentration of ionized cytosolic Ca ions directly mediates the effect of glucose to stimulate insulin release from rat islet of Langerhans. Any alterations in calcium flux can have adverse effects on β-cell secretory function (Siddiqui *et al.,* 2014). A daily intake of 1,000–1,500 mg of calcium, especially in older subjects with diabetes, is recommended. This recommendation is safe and reduces osteoporosis in older persons. The value of calcium supplementation in younger persons is uncertain. Calcium improves insulin sensitivity in some type 2 diabetic populations (Rajendra *et al.,* 2007). In addition, calcium is essential for insulin-mediated intracellular processes in insulin-responsive tissues such as skeletal muscle and adipose tissue; any alteration in calcium may

contribute to peripheral insulin resistance via impaired insulin signal transduction, leading to decreased glucose transporter 4 (GLUT4) activities (Siddiqui *et al.,* 2014).

2.9.5 Phytochemicals and diabetes mellitus

2.9.5.1 Antidiabetic phytoconstituents

The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most impotant of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds (Mir *et al.,* 2013). Constituents that come under the category of polysaccharides, peptides alkaloids, glycopeptides, triterpeniods, lipids, amino acids steroids, xanthone and guanidines phenolics, coumarins, iridoids, alkyldisulphides flavonoids and inorganic ions are reported to have antidiabetic activity (Jaraid *et al.,* 2008).

2.9.5.1.1 Alkaloids

The major alkaloid component that is known to suppress hyperglycemia is alkaloid I-ephedrine. It has demonstrated to promote the regeneration of pancreatic islets cells following atrophy, restores insulin secretion, and thus reduces hyperglycemia (Elliot *et al.,* 2000). Hydrolysis of Alkaloids like pyrrolizidine alkaloids (PAS) via N-oxidation and demethylation in the liver leads to intense cellular alterations known as megalocytosis (Zeinsteger *et* al., 2008). Alkaloids lead to fatty degeneration, proliferation of the biliary tract epithelium, megalocytosis, liver cirrhosis, nodular hyperplasia and adenomas or carcinomas (Zeinsteger *et* al., 2008).

2.9.5.1.2 Flavanoids

Flavanoid glycosides constituents that have been used in clinical treatment of diabetes to improve sensitivity of insulin include strictinin, isostrictinin and pedunculagin (Zhou, 2009). Flavonoids can be widely classified into different categories like flavonols, flavones, catechins and flavones. Quercetin is an impotant flavanoid known to posses a vast array of pharmacological activities (Mukherjee, 2006). Some flavanoids such as kakonein, flavone C-glycoside and caesalpin P have antidiabetic activity (Li *et al*., 1999) and most of them improved the function of pancreatic islet cells. The polyhydroxylated flavonol myricetin demonstrated insulinomimetic properties stimulating lipogenesis and glucose transport in the adipocytes. This compound has no effect on insulin receptor autophosphorylation or glucose uptake (Elliot *et al.*, 2000).

2.9.5.1.3 Saponins

Saponins are glycosides of triterpenes and steroids and commonly occur in higher plants. The sugars found in saponins are hexoses, 6-deoxyhexoses, pentoses, uronic acids, or amino sugars (Yang, 2009). Examples of saponins include triterpenoids a steroidal glycosides, they are found naturaly in many plants and are known to posse's potent hypoglycemic activity (Mukherjee, 2006). These saponins directly and/or indirectly regulate the activity of enzymes related to glucose metabolism. Saponins are poorly absorbed in the gastrointestinal tract and its overdose induces bloody diarrhea. Saponins are strong laxatives and in the small intestines, toxic doses lead to acute erosion of the superficial or middle parts of the intestinal villi resulting in haemorrhage inside the lamina propia (Yang, 2009). It also causes hemorrhage in many liver lobules and congestion of central veins and

liver sinusoids. In the kidneys, saponins results in haemorrage in the glomeruli and focal destruction of the renal tubules. The toxic level of saponins therefore, causes cardiac failure, acute hypoglycemia and hepatorenal damage leading to sudden death (Diwan, 2006).

2.9.5.1.4 Steroids

It is one of the familiar compounds occurring naturally in plants and animals, possessing a nuclear of cyclopentanohydropheranthrene, sterols bile acid steroid hormones, cardiac glycosides, toad poison; steroidal saponins are some impotant steroids (Yang, 2009).

2.9.5.1.5 Tannins

Tannins are polyphenols that are obtained from various parts of different plants belonging to multiple species. They are found in abundance in the tree bark, wood, fruit, roots, fruit pod, leaves, and also in plant gall. Tannins can be classified into two broad groups – hydrolysable tannins and condensed tannins. Clinically all the forms of tannin may participate in managing glucose level in blood (Kumari, 2014a**).**

2.9.6 Herbal management of diabetes mellitus

Medicinal plants, since times immemorial, have been used in virtually all cultures as a source of medicine.Traditional medicine, in the estimate of the World Health Organization is used by up to 80% of the population of most developing countries (WHO, 2002). Herbal medicines are usually widely perceived by the public as being natural, healthful and free from side effects, but that is an over simplification

of the matter. The chemical composition of herbal products and potency depends on the plant extract derivative, the age of the plant part used, season when harvested and the methods of processing (Yuan *et al.,* 2015).

Herbal medicines and traditional healers are receiving attention from mainstream health officials and international medical research and training institutions as governments confront the high cost and inefficiencies of official health programs (Srinivasan, 2005). In the management of diabetes mellitus some herbal alternatives have proven to provide symptomatic relief and assist in the prevention of the secondary complications of the disease. Others help in the regeneration of ß-cells; overcoming resistance; possess antioxidant activity and lowering of cholesterol action. Phytochemicals, which are secondary metabolites of medicinal plants help preserve cell function and prevent diabetes induced reactive oxygen species (ROS) formation (Nyamai *et al.,* 2016). A few examples of such plants include:

2.9.6.1 *Aloe vera*

The dried sap (fluid) of *Aloe vera* is a traditional remedy used for diabetes in the Arabian Peninsula. It has been reported to have hypoglycemic effect in both type II patients and in animal model (Grover, 2006). *Aloe vera* juice is prepared from *Aloe vera* gel, a mucilaginous preparation obtained from the leaves of the plant. Oral administration of the juice has been reported to reduce fasting blood glucose and triglyceride levels in type II diabetic patients with or without combination of a conventional anti-diabetic agent (Rashidi, 2013).

2.9.6.2 *Camellia sinensis* and *Acacia arabica*

Epigallocatechin gallate, present in *camellia sinensis* increases insulin activity and prevents oxidative damages in streptozoan induced diabetic rats. Lower dose of *camellia sinensis* on SD rats fed with high fat diet for two weeks showed insullinotropic effect in experimental condition (Patel *et al.,* 2012).

Acacia arabica is found throughout the drier parts of India, the bark of this plant has various uses, and it is reported for significant hypoglycemic activity (Mukherjee, 2006). The chloroform extracts of *A. arabica* bark in diabetic at 250 and 500mg/kg body weight for two weeks, significantly decreased the serum glucose level and resistance total cholesterol, triglyceride high density lipopoprotein (HDL) and low density lipopoprotein (LDL) level (Rashidi, 2013).

2.9.6.3 *Artemisia parlens*

It is a shrub used in the treatment of DM in the southern part of India. The hypoglycemic activity of the methanol extract of the aerial parts of artemisia pallens has been reported (Mukherjee, 2006).

Allium cepa commonly known as onion, studies indicate the hypoglycemic activity of this plant along with its hypolipidemic and antioxidant activity. Investigations revealed the presence of a sulphur containing amino acid, smethylcysteine sulphoxide; in onion which was shown to impart potent hypoglycemic activity when administered at a dose of 200mg/kg for 45 days to alloxan induced diabetic rats (Mukherjee, 2006; Wadkar *et al.,* 2008).

2.9.6.4 *Ginseng species*

The *Panax ginseng* (Asian ginseng) and *Panax quinquefolius* (American ginseng) are the most commonly used ginseng species because of their constituents that include ginsenosides, polysaccharides, peptides, polyacetylenic alcohol, and fatty acids (Xie *et al.*, 2004). The chemical composition of *ginseng* products and potency varies with the plant extract derivative, the age of the roots, the location where grown, the season when harvested, and the methods of drying and extraction (Yuan *et al.*, 2008). The most commonly reported side effects of ginseng are nervousness and excitation, but these diminish with continued use or dosage reduction. *Ginseng* may exert an estrogen-like effect in postmenopausal women, resulting in diffuse mammary nodularity and vaginal bleeding (Jian, 2009). *Ginseng* may inhibit the effects of warfarin. It may interact with the monoamine oxidase inhibitor phenelzine (Jian, 2009).

2.9.6.5 *Ocimum sanctum*

Ocimum sanctum has been reported to contain various alkaloids, glycosides, tannins, saponins and many other compounds, which remain to be identified. Oral administration of alcoholic extract of the plant led to a marked lowering of blood sugar in normal glucose fed hyperglycemic and streptozotocin induced diabetic rats (Shanthy *et al.*, 2014). Further, the extract potentiated the extract of exogenous insulin in normal rats. The efficacy of *O. sactum* in reducing the increased blood sugar level in immobilized stress conditions with the oral administration of extract in albino rats has been determined (Shanthy *et al.*, 2014).

2.9.6.6 *Momordica charantia* (Bitter melon)

Momordica charantia, also known as bitter melon is widely cultivated in Asia, Africa, and South America extensively used in folk medicines as a remedy for diabetes mellitus. The antihyperglycemic effect has been demostrated in animal experimental models as well as human clinical trials from the fresh juice or unripe fruit of the *Momordica charantia* (Ibraheem *et al.,* 2012). Charantin an alcohol-extracted constituent of *Momordica charantia* consists of mixed steroids and in an animal study it was found to be more potent than the oral hypoglycemic agent tolbutamide (Sarkar *et al.,* 2008). Bitter melon also exhibits an insulin-like polypeptide, polypeptide-P, similar in structure to bovine insulin decreasing blood sugar levels when injected subcutaneously into type 1 diabetics (Baldwa *et al.,* 1997). The herb also has demonstrated to inhibit gluconeogenesis process (Shih *et al.,* 2006).

2.9.7 Review of plant species under study

2.9.7.1 *Croton macrostachyus*

Croton macrostachyus is a deciduous tree 3-25 m high with an open rounded crown and large spreading branches (Orwa *et al.,* 2009). It is common in secondary forests, on forest edges along rivers, around lakes, in moist or dry evergreen upland forests, woodlands, wooded grasslands or clump bushland and along roadsides. Outside the forests, in wetter areas, the species is widely distributed. The bark is pale gray or gray-brown, finely reticulate, fairly smooth, and finely fissured with age. The shoots are dense and hairy. Leaves are large, green, turning to orange before falling, ovate, base is subcordate or rounded, apex acuminate, margin crenulate-serrulate or subentire, stellate hairy but more densely so beneath on long

stems crowded at the ends of branchlets; veins prominent with 2 stalked glands just visible at the base of the leaf, paler below due to soft hairs; texture more or less furry and margin slightly toothed (Orwa *et al.,* 2009).

Boiled leaf decoction of *Croton macrostachyus* is drunk or ashes taken orally as treatment for cough; juice from fresh leaves is applied on wounds to hasten clotting. Root decoction is used as an anthelmintic for tapeworm, as a purgative, and for malaria and venereal diseases. Bark from the stems and roots is boiled in water and newly born babies are bathed in the mixture as a remedy for skin rash. Seeds and resin are poisonous. Leaves can be used as fodder (Orwa *et al.,* 2009).

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Plate 2.1: *Croton macrostachyus* (photograph taken in July 2013, at Kijauri Nyamira County)

2.9.7.2 *Azadiratchta indica*

The NEEM tree (*Azadiratchta indica*) is a tropical evergreen and fast growing tree native to India where it is known as "the village pharmacy" because of its healing versatility. Botanically, it belongs to the family *Meliaceae* (Tomar *et al.,* 2011).

It has a straight trunk and the tree can reach a height of 30 meters and more, though 15 to 20 metres is the average. The bark is hard, rough and scaly, fissured even in young trees. It is often brown, but in older trees it can be pale or greyish-black. Spreading branches with dark green leaves form a dense, round canopy, up to 20 metres across. (It makes a very good shade tree). Leaves are alternate (not opposite each other along the branches) and consist of several leaflets with serrated edges. The flowers are large clusters of up to 250 individual white blooms. The whole cluster can be quite large, but the individual flowers are only a few mm across. They look inconspicuous; however, the scent is just heavenly. You can smell a flowering neem tree from miles away. The olive like, edible fruit is oval to round and thin skinned (edible does not mean tasty). It is green initially and turns yellow when ripe. Every fruit contains one, and sometimes two or three, seeds (Tomar *et al.,* 2011).

Neem is easy to grow in a wide range of temperatures and conditions, and the tree can live for 150 to 200 years. All parts of the neem plant have been used for many centuries in India. From India the tree and the knowledge about its many uses and benefits have spread. Today neem grows in many countries across the world, and wide range of neem products is available commercially. The seeds bark and leaves contain compounds with proven antiseptic, antiviral, antipyretic, anti-inflammatory, anti-ulcer and antifungal uses (Tomar *et al.,* 2011).

Some of the most important documented uses of various parts of the neem tree are:

i. **Neem oil** is extracted from the seeds of the neem tree and has insecticidal and medicinal properties for which it has been used for thousands of years in pest control, cosmetics, medicines (Debjit *et al.,* 2010).

ii. **Neem leaves** are used to treat chickenpox and warts by directly applying to the skin in a paste form or by bathing in water with neem leaves. In order to increase immunity of the body, neem leaves are also taken orally in the form of neem capsules or made into a tea. The tea is traditionally taken orally to reduce fever caused by malaria. This tea is extremely bitter. It is also used to soak feet for treating various foot fungi. It has also been reported to work against termites. In Ayurveda, neem leaves are used in curing neuromuscular pains. Neem leaves are also used in storage of grains inhibiting nitrification. It also works as a nematicide (Tomar *et al.,* 2011).

iii. **Twigs of neem** are also used in India and Africa as toothbrushes. Nowadays toothpastes with neem extracts are also available commercially (Debjit *et al.,* 2010).

iv. **Neem (leaf and seed) extracts** have been found to be spermicidal and thus research is being conducted to use neem extracts for making contraceptives. Neem produces pain relieving, anti-inflammatory and fever reducing compounds that can aid in the healing of cuts, burns, earaches, sprains and headaches, as well as fevers (Debjit *et al.,* 2010).

v. **Neem bark and roots** in powdered form are also used to control fleas & ticks on pets. Neem has **anti-bacterial properties** that help in fighting against skin infections such as acne, psoriasis, scabies, eczema, etc. Neem

extracts also help in treating diabetes, AIDS, cancer, heart disease, herpes, allergies, ulcers, hepatitis and several other diseases (Tomar *et al.,* 2011).

Plate 2.2: *Azadiratchta indica* (photograph taken in July 2013 at Kijauri Nyamira County)

2.9.7.3 *Lippia javanica* (The Lemon Bush)

The Lemon Bush is an erect, multi-branched, woody shrub that grows 1-2 m tall. Stems are more or less square in cross-section. The 3-4 cm-long leaves are hairy on sides and have dentate, lightly toothed, margins, and are rough to the feel with deeply sunken veins from above. Leaves are opposite, often in whorls of up to four and have a characteristic lemon scent when crushed. Small creamy white flowers clustered together in dense, round spikes about 1 cm in diameter are produced between February and May (but can be found throughout the year). Seeds are small brown nutlets (Viljoen *et al.,* 2005).

This plant is well known medicinally in many African tribes and to many avid herbalists. The leaves (and stems) are made into a tea as a cough and cold remedy,

to bring down fevers and to treat malaria. It is also excellent for treating skin problems, scabies and scalp infections. Some people inhale the smoke for asthma and chronic cough. Preparations are also used as an anti-inflammatory to soothe sore muscles. Xhosa people have used Lemon Bush to disinfect meat that has been contaminated with anthrax. It is also used traditionally as a charm for protection against dogs, lightning and crocodiles and for ritual cleansing after contact with a corpse (Viljoen *et al.*, 2005).

Clinical studies using human volunteers have also shown that Lemon Bush extract is a more potent malaria vector mosquito repellant than most available commercial formulation. The plant is also used as an infusion for fevers, 'flu, coughs, colds and chest complaints. It is applied topically to treat skin rashes (Viljoen *et al.*, 2005).

Plate 2.3: *Lippia javanica* (photograph taken in July 2013, at Kijauri Nyamira County)

2.9.7.4 *Ocimum lamiifolium*

Genus Ocimum is widespread over Asia, Africa, Central and Southern America. The genus Ocimum is cultivated for its extraordinary essential oil which display many therapeutic usages such as in medicinal application, herbs, culinary, perfume for herbal toiletries, aromatherapy treatment and as flavouring agent (Ekunwe *et al.,* 2010). It is perennial evergreen shrub having oblong, ovate green coloured leaves (0.5-5 m), oppositely arranged having pubescent leaf surface, narrow at the base and deeply serrated. One seeded fruits are indehiscent type found in clusters, hermaphrodite flowers are found in clusters, tap roots are deep and soft wooded (Kumar *et al.,* 2010). It has wide range of therapeutic effects like antimicrobial, antispasmodic, bactericide, carminative, anthelmintic, hepatoprotective, antiviral, larvicidal, remedy of coughs, colds, measles, abdominal pains, diarrhoea, insect repellent, particularly against mosquitoes and storage pest control (Ekunwe *et al.,* 2010).

Plate 2.4: *Ocimum lamiifolium* (photograph taken in July 2013 at Kijauri Nyamira County)

2.9.7.5 *Persea americana*

Persea americana is a medium to large tree, 9-20 m in height. The avocado is classified as an evergreen, although some varieties lose their leaves for a short time before flowering. The tree canopy ranges from low, dense and symmetrical to upright and asymmetrical (Orwa *et al.,* 2009). Leaves are 7-41 cm in length and variable in shape (elliptic, oval, and lanceolate). They are often pubescent and reddish when young, becoming smooth, leathery, and dark green when mature. Flowers are yellowish green, and 1-1.3 cm in diameter. The many flowered inflorescences are borne in a pseudo-terminal position. The central axis of the inflorescence terminates in a shoot. The fruit is a berry, consisting of a single large seed, surrounded by a buttery pulp. It contains 3-30% oil (Florida varieties range from 3-15%). The skin is variable in thickness and texture. Fruit colour at maturity is green, black, purple or reddish, depending on variety. Fruit shape ranges from spherical to pyriform, and weigh up to 2.3 kg (Orwa *et al.,* 2009).

Some hybrid varieties are best adapted to a lowland tropical climate and relatively frost-free areas of the subtropics. The tree is grown for its nutritious fruit that has long been important in the diets. Consumption is most often as an uncooked savoury dish mixed with herbs and/or spices, as an ingredient of vegetable salads, or as a sweetened dessert. However, its texture and colour can be used to enhance the presentation and consumption of many foods. Cooking impairs flavour and appearance of avocados (Orwa *et al.,* 2009).

The pulp and the seeds contain fatty acids, such as oleic, lanolic, palmitic, stearic, linoleic, capric and miristic acid which constitutes 80% of the fruits fatty content.

The oil is used by the cosmetic industry in soaps and skin moisturizer products. Watery extracts of the avocado leaves contain a yellowish-green essential oil. Recently anti-cancerous activity has been reported in extracts of leaves and fresh shoots of avocado. Oil extracted from the seeds has astringent properties, and an oral infusion of the leaves is used to treat dysentery. The skin of the fruit has anti-helmintic properties. The avocado is also said to have spasmolitic and abortive properties. The seed is ground and made into an ointment used to treat various skin afflictions, such as scabies, purulent wounds, lesions of the scalp and dandruff. The flesh is also used in traditional medicine. The unripe fruit is poisonous and the ground-up seed mixed with cheese is used as a rat and mouse poison (Orwa *et al.*, 2009).

Plate 2.5: *Persea americana* (photograph taken in July 2013, at Kijauri Nyamira County)

2.10 Biochemical markers of i*n vivo* toxicity

2.10.1 Alkaline phosphatase (ALP)

Alkaline phosphatase is an enzyme that catalyzes specific chemical reactions within the body. However, if it is present in serum in large amounts, it is diagnostic of bone or liver disease or a tumor in these organs (Decie and Lewis, 1991). Medically, ALP is found in the liver, bone, placenta, and intestine. A healthy liver continually drain away fluid containing ALP and other substances through the bile duct when the liver is diseased the bile duct may be blocked accumulating this fluid which eventually escapes into the blood stream. Usually the cells lining the bile ducts in the liver produce the ALP as the first enzyme if the liver disease is primarily of an obstructive nature (Cholestatic). If the disease on the other hand, is primarily of the liver cells (hepatocytes); the aminotransferases rise prominently in blood. Therefore, these diagnostic enzymes are useful in distinguishing the type of liver disease, either cholestatic or hepatocellular (Fischbach, 1996).

2.10.2 Aspartate aminotransferase (AST)

It is a cytoplasmic and mitochondrial enzyme predominantly found in the liver, heart, skeletal muscles, kidney, pancreas, erythrocytes, lungs and brain tissue (Warnes, 1982). It is a biomarker for a diseased state or injury to these tissues. The amount of AST found in the blood stream corresponds with the number of cells affected by the disease or injury, but the level of elevation depends on the length of time that the blood is tested following the injury. After cell injury, serum AST levels become elevated within eight hours and reach the peak at 24-36 hours, and returns to normal in three to seven days. In case of chronic (ongoing) cell injury, AST levels remain elevated (Warnes, 1982). AST is a valuable biomarker of liver

disease and is used in combination with other enzymes, for example, alanine aminotransferase (ALT); to monitor the cause of various liver disorders. AST is also diagnostic of other disorders or diseases such as acute pancreatitis, muscle disease, trauma, severe burn and infectious mononucleosis (Knottnerus, 2015).

2.10.3 Alanine aminotransferase (ALT)

It is a cytoplasmic enzyme predominantly found in the liver and to a lesser extent in skeletal muscles, kidney and heart (Zamora et al., 1996). A rise in plasma ALT activities is indicative an injury to the cytoplasmic membrane in the cell. It is a specific biomarker for cytoplasmic and mitochondrial hepatic injuries where its high concentration is greater than that of AST (Zamora *et al*., 1996).

2.10.4 Creatinine kinase (CK)

Creatine kinase is the enzyme responsible for regeneration of ATP (Hearse, 2014). 95% of the CK present in normal serum is of skeletal muscle origin probably results due to leakage from skeletal muscle, particularly during physical activity. Healthy and active persons show higher values of serum CK activity. Moreover, CK values are lower in women than men and are usually lower in the morning than in the evening. Hospitalized patients tend to have lower values, possibly because bed rest decreases the amount of enzyme released from skeletal muscle (Kaplan, 1989). Reference interval for total CK activity lies between 130-253 µ/L in humans (Kaplan, 1989). Such wide variation occurs due to age, sex and race and in normal human subjects, only high increases in CK activity are clinically significant (Kaneko, 1989).

2.10.5 Blood urea nitrogen (BUN)

Urea is synthesized in the liver from amino acids resulting from protein metabolism and it is a major pathway of nitrogen excretion in humans. Urea is released in the blood and cleared by the kidneys. The source of Protein results mainly from diet or tissues and the rate of urea production is potentiated by a high protein diet, tissue damage or sepsis and increased catabolism due to starvation. Blood urea nitrogen (BUN) is used as a biomarker of glomerular function if 60% of glomeruli is destroyed (Wardner, 1985). Low blood urea nitrogen is observed following over-administration of intravenous infusion, appropriate antidiuretic hormone (ADH) secretion or during pregnancy (the most common cause in young women) as a result of increased glomerular filtration rate (GFR). In children, low levels of urea are as a result of decreased synthesis of proteins where amino acids are used for protein anabolism during growth, low protein intake, very severe liver disease and inborn errors of urea cycle (Joan *et al.,* 1988).

2.11 Hematological markers of *in vivo* toxicity

2.11.1 Red blood cells

Red blood cells (erythrocytes) are anucleate cells that are packed with the oxygen-carrying protein, hemoglobin. Under normal conditions, the concentration of erythrocytes in blood is approximately 3.9-5.5 million/μL in women and 4.1-6 million/μL in men (Junquiera *et al.,* 1992). A decrease in number of red cells in the blood is often associated with development of anemia (Junquiera *et al.,* 1992). A primary increase in bone marrow activity causes polycythaemia (erythrocytosis) This occurs due to the myeloproliferative disorder or in response to increased erythropoietin production either as a consequence of chronic hypoxaemia or

because of inappropriate erythropoietin secretion, especially the lung or renal disorders (Haslett *et al.,* 2002).

2.11.2 Hemoglobin

Hemoglobin is the oxygen carrying protein found in the red blood cells and it is synthesized in the bone marrow. Low levels of hemoglobin as a result of loss of blood (hemorrhage) or immature reticulocytes usually related to iron deficiency in the diet; or accelerated blood cell destruction leads to anemia (Junquiera *et al.,* 1992). Alterations in hemoglobin molecules also results in sickle cell disease. Sickle cell disease is characterised by viscous sickle shaped erythrocytes that become rigid with a shortened life span often leading to profound anemia (Junquiera *et al.,* 1992).

2.11.3 White blood cells

White blood cells (leukocytes) provide immunity to the body against antigen invasion. They are classified on the basis of the type of granule in their cytoplasm and the shape of the nucleus, into two groups: granulocytes (polymorphonuclear leukocytes) and agranulocytes (mononuclear leukocytes). Neutrophils, eosinophils, and basophils are granulocytes (terminal cells) while agranulocytes include the lymphocytes and monocytes which can also undergo through several cycles of activity before dying (Junquiera *et al.,* 1992).

2.11.3.1 Neutrophils

Neutrophils are polymorphonuclear leukocytes constituting 60-70% of circulating leukocytes in humans. They are short-lived cells with a half-life of 6-7 hours in

blood and a life span of 1-4 days in connective tissues. After which they then die, whether or not they have engaged in phagocytosis activity; hence termed as terminal cells. The neutrophils defend the body against invasion by microorganisms, especially bacteria (Junquiera *et al.,* 1992). Clinically, an increase in neutrophils in the blood (neutrophil 'leucocytosis' or 'neutrophilia') is usually as a result of an infection and tissue injury. Reduced levels of neutrophils in the blood (neutropenia) are seen in a wide range of inherited and acquired disorders like HIV infection (Horward *et al.,* 2002).

2.11.3.2 Monocytes

Monocytes are agranulocytes found in the blood representing the recently formed precursors of the mononuclear phagocyte system. Monocytes differentiate into macrophages after they enter connective tissues and interact with lymphocytes and play an essential role in the recognition and interaction of immunocompetent cells and antigen (Junquiera *et al.,* 1992). The half-life of the monocyte in blood is 12-100 hours. Chronic bacterial infections such as tuberculosis, inflammation and malignant disorders result in monocytosis while corticosteroid treatment is often associated with monocytopenia (Horward *et al.,* 2002).

2.11.3.3 Lymphocytes

Lymphocytes constitute B and T lymphocytes. T lymphocytes make up 75% of the lymphocytes and are the cells of the cell-mediated response whilst B lymphocytes releases antibody against a specific antigen (humoral immunity). T lymphocytes are less autonomous than B lymphocytes and interact with antigen presenting cells

expressing self-histocompatibility molecules human leucocyte antigens (HLA) for the recognition of the antigen by the T cell receptor (TCR) (Horward *et al.,* 2002).

B lymphocytes are responsible for humoral immunity and following an appropriate antigenic stimulus, they transform into plasma cells and secrete antibody specific to that antigen (Horward *et al.,* 2002). Within the lymphoid tissues, such as lymph nodes and spleen, B cells undergo a morphological transformation into immunoblasts and, ultimately, plasma cells upon stimulation by an antigen (Horward *et al.,* 2002).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection and preparation of plant materials

The plants used in this study were collected from their native habitats on the basis of ethnobotanical information. They were collected with bioconservation aspects in mind from Kijauri village Nyamira county Kenya. Information on the identity of the plant to collect, the precise locality where it grows, what part to collect, when curative potency is at maximum and the mode of preparation was provided by a traditional medical practitioner. For this study, the parts of the plants collected were the leaves. Botanical identities of the plants were authenticated by an acknowledged authority in taxonomy and a voucher specimen deposited at the National Museums of Kenya Herbarium, Nairobi. Voucher specimens deposited at the National Museums of Kenya were assigned voucher numbers as WM01 (*Croton macrostachyus),* WM02 (*Azadiratchta indica),* WM03 (*Lippia javanica),* WM04 (*Ocimum lamiifolium),* and WM05 (*Persea americana).*

Leaves were collected while green and dried at room temperature away from direct sunlight for different periods of time depending on their succulence. The dried leaves were separately ground into fine powder by use of an electric mill. The powdered plant materials were kept at room temperature away from direct sunlight in closed, dry plastic air tight bags ready for extraction.

3.2 Extraction

One hundred grams (100g) of each powdered plant material was later extracted in 1 liter of distilled water at 60°C in a metabolic shaker for 6 hours. The extracts were left to cool, decanted into a clean dry conical flask and then filtered through folded cotton gauze into another clean dry conical flask. The filtrates were stored in a refrigerater at 4ºC. The filtrates were then freeze dried using a Modulyo Freeze Dryer (Edward England) in 200ml portions for 48 hours. The freeze-dried powder was then weighed and stored in an airtight container at -20^0 C until used for bioassay.

3.3 Preparation of extracts doses for bioassays

The appropriate doses of freeze-dried plant extracts were prepared by dissolving 0.012g in 2ml (25mg/kg body weight), 0.022g in 2ml (48.4mg/kg body weight), 0.043g in 2ml (93.5mg/kg body weight), 0.084g in 2ml (180.9mg/kg body weight) and 0.162g in 2ml (350mg/kg body weight). Insulin dose was prepared by dissolving 12 insulin units in 10 ml (1IU/kg body weight) of physiological saline while glibenclamide dose was prepared by dissolving 6.9mg in 10ml (3mg/kg body weight) of 0.1ml physiological saline. The plant extract solution of 0.1ml was administered to all groups of the animals. All the drug solutions were stored at -20ºC until used.

3.4 Laboratory animals and experimental design

The study used healthy adult male Swiss albino mice (3-5 weeks old) that weighed 20-26g with a mean weight of 23g. The animals were allowed to acclimatize for a period of two weeks in the animal house at the department of Biochemistry and

Biotechnology, Kenyatta University prior to study. The mice were housed in polypropylene cages, maintained at a temperature of 25^0C with 12 hours/12 hours darkness photoperiod and fed on rodent pellets and water *ad libitum*. The Principles of Laboratory Animal Care (Hau *et al.*, 2002) were followed.

Activity of aqueous extracts was studied in alloxan-induced diabetic mice. The mice were divided into two portions for each plant extract. The first portion was used for antidiabetic assay through intraperitoneal administration of aqueous plant extract. It consisted of the following groups of five mice each; Group I consisted of normal un-manipulated mice (the reference group of the experiment treated with 0.1ml physiological saline; Group II consisted of alloxan-diabetic negative control mice (treated with 0.1ml physiological saline alone (vehicle); Group III consisted of alloxan-diabetic positive control mice treated with insulin (at 1 IU/kg body weight); Group IVa consisted of alloxan-diabetic experimental mice treated with 25 mg/kg body weight of aqueous plant extract; Group IVb consisted of alloxan-diabetic experimental mice treated with 48.4 mg/kg body weight of aqueous plant extract; Group IVc consisted of alloxan-diabetic experimental mice treated with 93.5 mg/kg body weight of aqueous plant extract; Group IVd consisted of alloxan-diabetic experimental mice treated with 180.9 mg/kg body weight of aqueous plant extract; and Group IVe consisted of alloxan-diabetic experimental mice treated with 350 mg/kg body weight of aqueous plant extract.

The second portion was used for antidiabetic assay through oral administration of aqueous plant extract. The experimental design was similar to the first portion

except that the reference oral drug used was glibenclamide (at 3mg/kg body weight).

3.5 Induction of hyperglycemia

Hyperglycemia was induced experimentally by intraperitoneal administration of alloxan monohydrate obtained from Sigma (Steinhein, Switzerland). Alloxan monohydrate acts by causing specific necrosis of pancreatic islets (Szkudelski, 2001). The animals were injected intraperitoneally with 186.9 mg/kg body weight of 10% alloxan monohydrate. Three days after administration of alloxan, blood glucose level were measured with a glucose analyzer model (Hypogaurd, Woodbridge, England). Mice with blood glucose levels above 200mg/dL (>11.1mmol/L) were considered diabetic. They were fasted for 8-12 hours prior to use in bioassay (Szkudelski, 2001) but allowed free access to water until the end of the experiment.

3.6 Blood sampling

Blood sampling was carried out by caudal vein tail bleeding. The tail was sterilized with 10% alcohol and then nipped at the start of the experiment and repeated after 1, 2, 3, 4, 7 and 24hrs. The blood glucose levels were determined every time with a glucose analyzer model (Hypoguard, Woodbridge, England).

3.7 *In vivo* toxicity evaluation

The mice were randomly divided into four different groups of five mice each. Group I and II served as the untreated control mice intraperitoneally and orally, respectively, administered with 0.1ml physiological saline daily for 28 days. The other two groups were treated with 1000 mg/kg body weight of the extract. The extracts were administered intraperitoneally for one group and orally for the other group on a daily basis for a period of 28 days. During this period, the mice were allowed free access to mice pellets and water *ad libitum*. The same experimental design described above was followed separately at different timeline with subsequent dosages of 450 and 670mg/kg body weight (Appendix 2.1). The mice were observed for signs of general illness, change in behavior and mortality.

3.7.1 Determination of body weight

The body weight of each mouse was assessed during the acclimatization period, once before commencement of dosing, once weekly during the dosing period and once on the day of sacrifice.

3.7.2 Absolute organ weight

On the day of sacrifice, all the animals were euthanized. Absolute weight of the following carefully dissected organs was taken: brain, liver, kidneys, lungs, spleen, testis and heart. These organ were then stored in 10% neutral buffered formalin. The relative organ to body weight was then calculated using the forfulae below:

$$\text{Relative organ to body weight} = \frac{\text{Actual weight of the organ}}{\text{Body weight of individual mice on day of sacrifice}} \times 100$$

3.7.3 Biochemical assays

3.7.3.1 Preparation of sera samples

At the end of the experimental period (28 days), all animals were exsanguinated and blood samples were drawn from the heart of each sacrificed mouse. The blood samples were collected in plastic test tubes and divided into two portions. One portion was used for determination of biochemical parameters. This portion was allowed to stand for 3hours to ensure complete clotting. The clotted blood samples were centrifuged at 3000 rpm for 10 minutes and clear serum was aspirated off, packed in eppendorf tubes and stored frozen at -20^0C. Olympus 640 chemistry auto analyzer was used. A number of events that occurred simultaneously were performed automatically under the direct control of the instrument microprocessor. All the assays were performed based on the standard operating procedures (SOPs) written and maintained in the Department of Laboratory Medicine, Kenyatta National Hospital.

3.7.3.2 Laboratory determination of enzyme activities and analytes

The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were determined according to the method by Henry *et al.* (1960). Creatine kinase was determined according to the Oliver and Rosalki method based on the primary reaction that is catalyzed by creatine kinase (CK) resulting in production of creatine and ATP. Finally, the blood level of urea (BUN) was determined according to Tiffany *et al*. (1992).

3.7.4 Determination of hematological parameters

The other portion of the sampled blood from the sacrificed mice was used for determination of hematological indices. The following hematological parameters were examined by standard protocols using coulter count system Beckman Coulter (Thermo Fisher, UK); red blood cells count, white blood cells, hemoglobin, mean cell hemoglobin concentration, packed cell volume (Jain, 1986). Air dried thin blood films stained with giemsa stain were examined microscopically using magnification x200 and x400 for differential WBC counts and cell morphologies, respectively. Neutrophil (N), lymphocyte (L), eosinophils (E), monocytes (M) and basophils (B) absolute counts (number of cells x 10^9) per liter were obtained by expressing their percent differential counts against the total WBC absolute counts (Jain, 1986).

3.7.5 Phytochemical analysis

The phytochemical screening of the plant extracts was qualitatively carried out to determine the class of secondary metabolites present. The presence of the following phytochemicals was investigated: alkaloids, saponins, tannins, terpenoids and sterols, flavanoids and anthroquinones. Standard methods described by Houghton and Raman (1998) and Hossein and Hani (2002) were used.

3.7.5.1 Test for alkaloids

A mixture of 2 g of each plant extract and 2 ml of 1% aqueous concentrated hydrochloric acid (HCl) was stirred and heated in a boiling water bath for 10 minutes. It was then filtered while hot and treated with Dragendorff's reagent.

Turbidity or precipitation was an indication of a preliminary test for alkaloids (Houghton and Raman, 1998; Hossein and Hani, 2002).

3.7.5.2 Test for sterols and terpenoids

N-hexane was stirred with 2 g of each extract to remove coloring materials. The residue was then extracted with 2 ml dichloromethane. The dicloromethane solution was dried over anhydrous sodium sulphate and mixed with 0.5 ml acetic anhydride followed by 2 drops of concentrated sulphuric acid. A gradual appearance of green to blue color was indicative of sterols. Color change from pink to purple indicated the presence of terpenoids (Houghton and Raman, 1998; Hossein and Hani, 2002).

3.7.5.3 Test for saponins

To 1g of the plant extract in 2 ml of distilled water in a test tube, a few drops of sodium bicarbonate solution were added and the mixture shaken vigorously. The occurrence of frothing which persisted for at least half an hour was taken as a positive test for saponins (Houghton and Raman, 1998; Hossein and Hani, 2002).

3.7.5.4 Test for flavanoids

Each of the 1 g of the plant extract was deffated by several washing in *n*-hexane. The deffated residue was washed with 4 ml 80% methanol and filtered. The filtrate was used as follows; to 2 ml of the filtrate, 1 ml of 1% aluminium chloride in methanol was added. The development of yellow color was an indication of the presence of flavanols, flavones and/or chalcones. In addition, to 2 ml of the filtrate, 1ml of potassium hydroxide was added. The development of a dark yellow color

was an indication of presence of flavanoids (Houghton and Raman, 1998; Hossein and Hani, 2002).

3.7.5.5 Test for tannins

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

3.7.5.6 Test for anthraquinones

The 2 g of the powdered plant materials was shaken with 10 ml of benzene and filtered. Five milliliters of a 10% ammonium hydroxide solution was added and the mixture shaken. The presence of a violet color in the ammonical phase was indicative of the presence of free anthraquinones.

Moreover, the plant extract material was washed in 2 g of benzene, boiled with 10 ml of 1% HCl and filtered while hot. The filtrate was shaken with 10 ml benzene. The benzene layer was removed and then 10% ammonia hydroxide added. The presence of a violet color was indicative of the presence of bound anthraquinones (Houghton and Raman, 1998; Hossein and Hani, 2002).

3.7.6 Elemental analysis

3.7.6.1 Total Reflection X-Ray Fluorescence (TXRF) System

The TXRF method employs a system where X-rays are made to impinge on the surface of a sample at glancing incidence such that total reflection occurs. The X-

rays excite atoms in the top layers of the material and the fluorescence is detected by a detector placed above the sample. The technique is sensitive to very dilute quantities of material. The X-ray source can be an X-ray tube or a synchrotron.This method was used to determine the content of manganese, iron, potassium, calcium, nickel, copper, zinc, strontium, bromine, molybdenum and lead in the aqueous extracts of the five selected medicinal plants.

3.7.6.1.1 Sample preparation

One gram (1g) of each sample was weighed into a clean vial (triplicates) and 10mL double distilled water added to each vial. Twenty microlitres (20µL) of 1000ppm Gallium stock solution was added into each sample (as internal standard) resulting in a concentration of 2ppm Ga in each sample. Each sample was mixed with a vortex mixer for one minute. Aliquots of 10µL of each sample were pipetted onto clean quartz carrier in triplicates and dried in an oven to evaporate the liquid.

3.7.6.1.2 Sample spectrum acquisition and quantitative analysis

Each sample carrier was irradiated for 1000 seconds using S2 Picofox TXRF Spectrometer which was operated at 50kV and a current of 1000µA with a molybdenum anode. Evaluation of the measured spectra was done using S2 Picofox software on the basis of the chosen elements. The concentrations were calculated based on the net intensities of the analyte peak elements and that of the internal standard as per the following formula;

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

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

Quantitative results obtained were copied to an excel worksheet. The worksheet was referred to as raw data. All the data in raw data worksheet was copied to edited worksheet where unnecessary data columns were deleted. Also, unnecessary element row lines were removed. Data in edited worksheet were further evaluated for averages and standard deviations for each set of sub-samples. Average and standard deviation functions in excel were used. Residual data was copied to final worksheet. To these data, all unnecessary columns were deleted leaving the calculated averages and standard deviations values as the final concentrations data (Hagen, 2007).

3.7.6.2 Atomic Absorbtion Spectrophotometry (AAS)

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

3.7.6.2.1 Preparation of reagents

3.7.6.2.1.1 Standard stock solutions

Chromium and magnesium standard stock solutions of 1000 ppm for AAS were used as supplied by the manufacturers (Aldrich Chemical Co., Inc), 1.7852g of vanadium pento-oxide was dissolved in minimum amount of concentrated

sulphuric acid and then heated to dissolve completely, then cooled. The solution obtained was adjusted to 1 litre with distilled deionised water. This gave 1000 ppm of the stock solution. Immediately each standard solution was prepared, the flask was thoroughly shaken for mixing and the contents then transferred into a clean plastic bottle and kept in a refrigerator.

3.7.6.2.1.2 Working standards

Suitable aliquots of standard stock solutions of each element were taken in a series of 100ml volumetric flasks. The solutions were diluted to volume using distilled deionised water, mixed thoroughly and transferred into plastic beakers. This procedure was done for each element when its analysis was due. During each analysis fresh working standards were prepared. For each element, working solutions were prepared within a given range where the relationship between the concentration and absorbance was linear. In case of magnesium, 2ml of 5% lanthanum solution was added to each series of working standards before diluting the standards to volume. In addition, standard blank reagents for Mg, V and Cr were prepared by adding all the used reagents, except the target element being determined.

3.7.6.2.1.3 Lanthanum solution

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

3.7.6.2.2 Digestion of the plant materials

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

3.7.6.2.3 Wet oxidation for determination of Cr, V and Mg

The dried samples weighing 1g were transferred into 100ml Pyrex beakers and to each beaker; 10ml of concentrated nitric acid was added, and then allowed to soak thoroughly. Three millilitres of perchloric acid (60% $HCL0_4$) was added to each beaker, and then warmed on a hot plate slowly, until frothing ceased. Heating was then intensified until all nitric acid was evaporated. When charring occurred, the mixture was cooled, 10ml of nitric acid was added and heating continued until white fumes of perchloric acid were observed. The final solution was then quantitatively transferred into 100ml volumetric flask by filtering through Whatman filter paper No. 42. The solutions were then made to volume and shaken well to allow proper mixing before the contents were transferred to plastic sample bottles. For each plant sample, digestion was done in duplicate. The samples were analyzed immediately whenever possible, otherwise kept in refrigerator at -20° C awaiting analyses.

3.7.6.2.4 Determination of Cr, V and Mg contents by atomic absorption spectroscopy (AAS)

The wet digests of the plant materials were analysed for Cr, V and Mg. The sample solutions for analysis of magnesium were prepared by withdrawing 1ml of the digested sample solution, into 100ml volumetric flasks. Five mililitres of lanthanum solution was added in each flask and the mixture diluted to volume using distilled deionised water. However, for analysis of Cr and V, the digested

sample solutions were analyzed without further dilution. After setting the AAS instrument to the right conditions for each element the respective standards and sample solutions were aspirated into the flame in turns to determine their respective absorbance. At least four standard solutions were aspirated between 6-10 samples to monitor the stability of the working conditions. Distilled deionised water was always flushed into the flame to re-establish the zero absorbance.

For each element, the above procedure was done in duplicate for each sample. The mean absorbance for each sample solution and standard solutions were calculated and recorded. The concentration values obtained were corrected by multiplying with the respective dilution factors. The final values were expressed as μg/g dry matter.

3.8 Data management and analysis

The data collected was entered into Ms Excel spread sheets where it was organised for statistical analysis. Analysis of data was done using SAS statistical software version 9.1.3. The results were expressed as mean ± standard deviation (SD). For efficacy studies, analysis of variance (ANOVA) and Tukey's post hoc tests were employed to compare means of untreated group of normal mice with alloxan induced diabetic group of mice treated with saline, conventional drug and the plant extracts. Evaluation of the safety was compared by testing the statistical significant difference among groups of control mice and those treated with the plant extracts at different doses using ANOVA and followed by Tukey's post hoc tests to separate the means and obtain the specific significant differences across treatments. The values of $p \leq 0.05$ were considered to be significant.

CHAPTER FOUR

RESULTS

4.1 *In vivo* hypoglycemic assay

4.1.1 *In vivo* hypoglycemic activity of *Croton macrostachyus*

The aqueous leaf extract of *Croton macrostachyus* yielded a 7% (w/w) light brown powder. Upon intraperitoneal administration, the extract lowered the blood glucose levels at all the five doses of 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight (Table 4.1). The effect was observed over three phases, whereby in the first hour, the extract caused a steep decline in blood glucose levels, followed by a steady decline from the second to seventh hour. A gradual increase was then observed in the twenty fourth hour. However, the reduction in sugar level was not in a dose related manner during the three phases. In the first hour, the extract lowered blood glucose levels to 82.1%, 90.3%, 55.3%, 75.4% and 71.2% for 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight doses, respectively, compared to insulin treated diabetic mice whose blood sugar levels were lowered to 50.2% within the first hour. By the third hour, all the five doses had lowered blood sugar levels to 44.1%, 46.4%, 41.5%, 49.7% and 44.0% respectively, compared to insulin treated diabetic mice whose sugar levels were lowered to 31.1% within the same hour (Figure 4.1).

Upon oral administration, the aqueous leaf extract of *C. macrostachyus* also lowered blood glucose levels at all the five doses of 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight from the first hour to the seventh hour in a dose-independent manner. A gradual increase was then observed from the seventh to the twenty fourth hours. However, by the second hour, the extract had lowered the blood glucose levels to 71.7%, 68.0%, 58.9%, 58.3% and 69.8% respectively for the five doses, compared to 50.2% for the conventional oral drug, glibenclamide (Figure

4.2). This was also a dose-independent response. The reduction in blood glucose levels when compared to the negative control was statistically significant ($p \leq 0.05$).

Table 4.1 Effects of intraperitoneally administered aqueous leaf extract of *Croton macrostachyus* on blood glucose levels in alloxan induced diabetic mice

	BLOOD GLUCOSE LEVELS AT VARYING TIMES (mmole/l)						
TREATMENT	**0hr**	**1hr**	**2hr**	**3hr**	**4hr**	**7hr**	**24hr**
Control/Saline	5.20±0.07^{c}	5.34±0.11^{e}	5.32±0.08^{d}	5.22±0.08^{b}	5.16±0.05^{b}	5.22±0.08^{b}	5.24±0.15^{c}
Diabetic/Saline	14.52±2.97^{ba}	16.02±2.48^{a}	17.52±2.62^{a}	19.20±2.88^{a}	21.22±3.23^{a}	22.50±3.48^{a}	25.00±2.98^{a}
Diabetic/insulin	17.46±2.99^{a}	8.62±0.55^{edc}	6.20±0.56^{cd}	9.32±0.29^{b}	4.96±0.11^{b}	4.70±0.28^{b}	6.30±0.79^{cb}
25(mg/kgbw)	15.42±1.26^{ba}	12.68±3.11^{bac}	8.90±1.64^{cb}	6.80±1.06^{b}	5.44±0.78^{b}	4.68±0.48^{b}	6.64±1.15^{cb}
48.4(mg/kgbw)	16.48±2.91^{ba}	14.98±3.49^{ba}	10.46±2.36^{b}	7.60±1.25^{b}	6.24±1.23^{b}	5.18±0.74^{b}	8.48±1.44^{b}
93.5(mg/kgbw)	13.68±2.55^{ba}	7.48±0.91^{ed}	6.30±0.46^{cd}	5.54±0.43^{b}	5.90±0.72^{b}	4.62±0.36^{b}	8.12±1.54^{cb}
180.9(mg/kgbw)	12.50±1.02^{b}	9.42±1.04^{adc}	7.28±0.98^{cbd}	6.16±0.23^{b}	5.48±0.34^{b}	5.02±0.15^{b}	7.84±0.59^{cb}
350(mg/kgbw)	15.96±3.33^{ba}	11.36±2.76^{bdc}	8.88±2.08^{cb}	6.94±1.30^{b}	5.68±0.98^{b}	5.14±0.70^{b}	6.64±0.55^{cb}

Results are expressed as Means ± SD for five mice per group. Values followed by the same superscript are not statistically different ($P \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test.

Table 4.2: Effects of orally administered aqueous leaf extract of *Croton macrostachyus* on blood glucose levels in alloxan induced diabetic mice

	BLOOD GLUCOSE LEVELS AT VARYING TIMES (mmole/l)						
TREATMENT	**0hr**	**1hr**	**2hr**	**3hr**	**4hr**	**7hr**	**24hr**
Control/Saline	5.16±0.11^{b}	5.22±0.08^{c}	5.18±0.08^{b}	5.22±0.08^{cb}	5.14±0.09^{b}	5.18±0.08^{b}	5.10±0.10^{c}
Diabetic/Saline	14.92±4.99^{a}	16.98±5.25^{a}	18.80±4.63^{a}	20.50±4.01^{a}	22.44±3.41^{a}	24.88±2.41^{a}	26.14±2.27^{a}
Diabetic/Glen	14.66±3.03^{a}	9.82±2.06^{bc}	7.38±1.88^{b}	5.96±0.93^{cb}	5.28±0.79^{b}	4.80±0.37^{b}	8.42±1.93^{b}
25(mg/kgbw)	12.96±3.59^{a}	12.44±4.84^{ba}	9.14±2.37^{b}	6.52±1.45^{cb}	5.02±0.88^{b}	4.80±0.86^{b}	10.04±1.62^{b}
48.4(mg/kgbw)	10.64±2.57^{ba}	9.72±5.36^{bc}	7.12±1.99^{b}	5.76±1.36^{cb}	5.08±1.38^{b}	4.44±1.67^{b}	8.88±2.08^{b}
93.5(mg/kgbw)	12.42±1.18^{a}	8.18±1.11^{bc}	7.32±0.75^{b}	6.24±0.65^{cb}	4.98±0.25^{b}	5.98±1.35^{b}	9.64±1.17^{b}
180.9(mg/kgbw)	9.14±1.11^{ba}	6.78±1.70^{bc}	5.32±1.19^{b}	4.20±1.11^{c}	4.44±0.90^{b}	4.68±0.83^{b}	7.76±1.23^{cb}
350(mg/kgbw)	12.72±3.47^{a}	9.66±2.74^{bc}	8.74±1.95^{b}	8.18±2.03^{b}	7.94±1.26^{b}	6.62±0.69^{b}	9.90±1.27^{b}

Results are expressed as Means ± SD for five mice per group. Values followed by the same superscript are not statistically different ($P \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test.

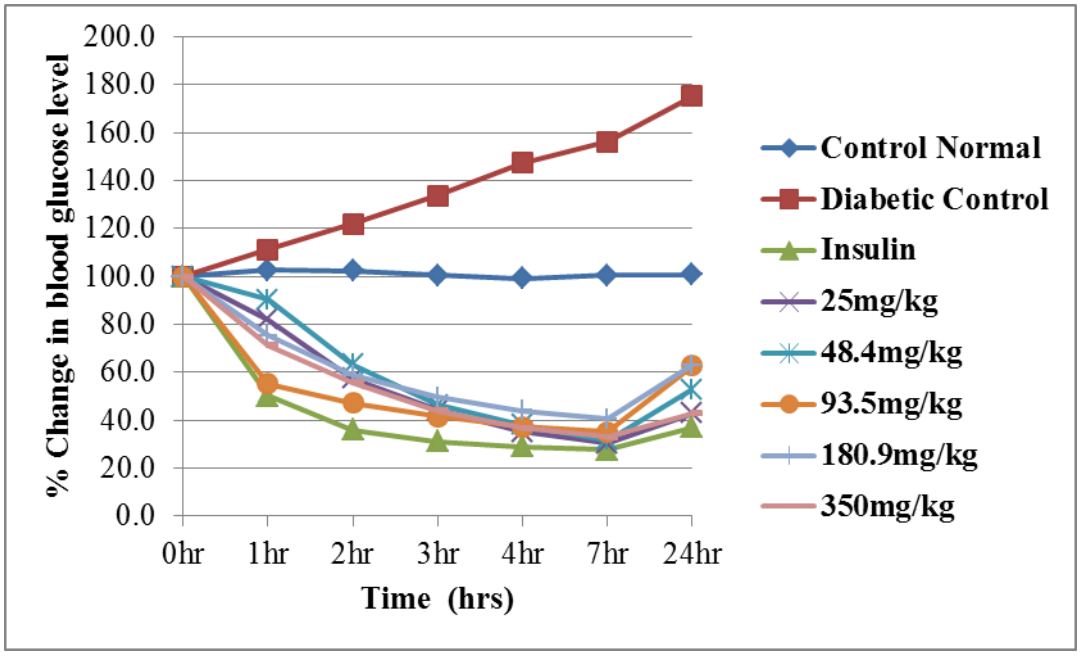

Figure 4.1: Mean percentage change in blood glucose levels of mice intraperitoneally administered with *Croton macrostachyus*

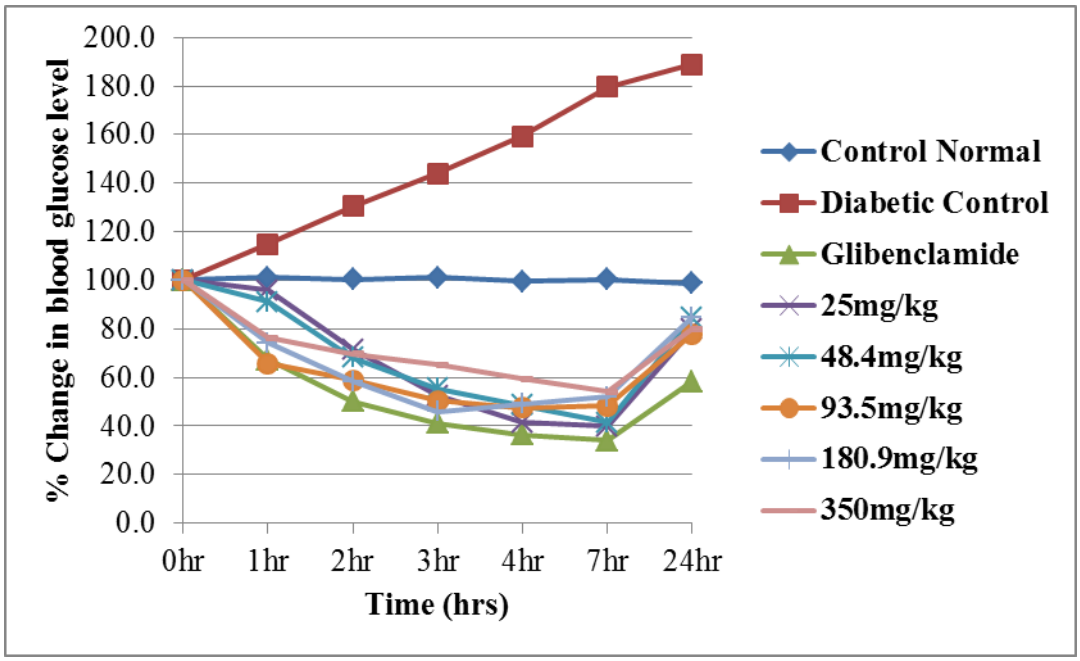

Figure 4.2: Mean percentage change in blood glucose levels of mice orally administered with *Croton macrostachyus*

4.1.2 *In vivo* hypoglycemic activity of *Azadiratchta indica*

Aqueous leaf extract yielded an 8% brown powder. Intraperitoneally, the extract lowered the blood glucose levels at all the five doses of 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight (Table 4.3). In the first hour, the extract caused a steep decline in blood glucose levels, followed by a steady decline from second to the seventh hour. In the twenty fourth hours, there was a gradual increase in blood glucose levels. However, the reduction in sugar level was not in a dose related manner. In the first hour, the extract lowered blood glucose levels to 59.1%, 70.6%, 56.8%, 61.2% and 71.4% for 25, 48.4, 93.5, 180.9 and 350mg/kg body weight doses, respectively, compared to insulin treated diabetic mice whose blood sugar levels was lowered to 50.3% within the first hour. By the fourth hour, all the five doses (25, 48.4, 93.5, 180.9 and 350 mg/kg body weight) had lowered blood sugar levels to 28.2%, 34.4%, 25.6%, 24.3% and 30.4%, respectively, compared to insulin treated diabetic mice whose blood sugar levels was lowered to 35.6% within the same hour (Figure 4.3).

Orally the extract of *A. indica* also lowered blood glucose levels at all the five doses of 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight (Table 4.4), from the first hour to the seventh hours in a dose-independent manner. By the second hour the extract had lowered the blood glucose levels to 40.2%, 48.2%, 47.7%, 44.4%, and 53.1% respectively for the five doses, compared to 56.6% for the conventional oral drug, glibenclamide (Figure 4.4). The reduction in blood glucose levels when compared to the negative control was statistically significant ($p \leq 0.05$).

Table 4.3: Effects of intraperitoneally administered aqueous leaf extract of *Azadiratchta indica* on blood glucose levels in alloxan induced diabetic mice

	BLOOD GLUCOSE LEVELS AT VARYING TIMES (mmole/l)						
TREATMENT	**0hr**	**1hr**	**2hr**	**3hr**	**4hr**	**7hr**	**24hr**
Control/Saline	5.24±0.11^{b}	5.24±0.54^{c}	5.20±0.10^{b}	5.16±0.05^{b}	5.12±0.08^{b}	5.14±0.09^{b}	5.16±0.09^{cd}
Diabetic/Saline	15.1±23.13^{a}	16.94±3.32^{a}	18.20±3.72^{a}	20.26±3.44^{a}	22.40±4.15^{a}	23.90±4.24^{a}	26.08±3.11^{a}
Diabetic/insulin	14.40±3.12^{a}	7.06±0.55^{cb}	5.94±0.40^{b}	5.14±0.11^{b}	4.92±0.08^{b}	4.56±0.21^{b}	7.40±0.61^{cb}
25(mg/kgbw)	17.84±3.78^{a}	10.32±1.59^{b}	7.00±1.33^{b}	5.70±0.97^{b}	4.94±0.75^{b}	4.40±0.58^{b}	7.52±0.72^{cb}
48.4(mg/kgbw)	13.42±3.00^{a}	9.50±2.33^{b}	6.60±1.19^{b}	5.38±0.86^{b}	4.54±0.61^{b}	3.50±0.50^{b}	8.32±1.02^{b}
93.5(mg/kgbw)	14.14±2.18^{a}	7.90±0.44^{cb}	5.78±0.59^{b}	4.52±0.54^{b}	3.60±0.60^{b}	2.78±0.49^{b}	6.22±0.89^{cbd}
180.9(mg/kgbw)	13.14±3.79^{a}	7.82±1.45^{cb}	5.54±0.90^{b}	4.12±0.75^{b}	3.12±0.74^{b}	2.08±0.43^{b}	5.16±0.74^{cd}
350(mg/kgbw)	12.96±2.27^{a}	9.10±1.28^{b}	7.24±1.44^{b}	4.90±0.93^{b}	3.86±0.52^{b}	3.12±0.66^{b}	4.18±0.74^{d}

Results are expressed as Means ± SD for five mice per group. Values followed by the same superscript are not statistically different (P≤0.05; analysed by ANOVA followed by Tukey's post hoc test.

Table 4.4: Effects of orally administered aqueous leaf extract of *Azadiratchta indica* on blood glucose levels in alloxan induced diabetic mice

	BLOOD GLUCOSE LEVELS AT VARYING TIMES (mmole/l)						
TREATMENT	**0hr**	**1hr**	**2hr**	**3hr**	**4hr**	**7hr**	**24hr**
Control/Saline	4.94±0.18^{b}	5.04±0.21^{c}	5.06±0.11^{c}	5.14±0.09^{cb}	5.12±0.08^{cb}	5.12±0.08^{b}	5.10±0.10^{b}
Diabetic/Saline	14.92±2.16^{a}	17.54±2.39^{a}	20.02±2.59^{a}	22.48±2.61^{a}	24.42±2.15^{a}	25.76±2.15^{a}	27.30±1.63^{a}
Diabetic/ Glen	14.44±3.08^{a}	10.88±2.94^{b}	8.12±1.58^{b}	6.70±1.18^{b}	5.14±0.55^{b}	4.98±0.34^{cb}	7.14±1.11^{b}
25(mg/kgbw)	14.56±0.57^{a}	9.68±1.19^{b}	5.86±0.79^{cb}	3.88±0.80^{c}	3.70±0.46^{cb}	3.36±0.40^{cbd}	6.44±0.99^{b}
48.4(mg/kgbw)	14.18±2.33^{a}	9.86±1.11^{b}	6.76±0.73^{cb}	4.28±0.43^{cb}	4.30±0.45^{cb}	4.22±0.79^{cbd}	7.96±1.98^{b}
93.5(mg/kgbw)	13.94±0.74^{a}	9.78±1.03^{b}	6.68±1.54^{cb}	3.40±0.79^{c}	3.06±0.86^{cb}	2.80±0.75^{d}	6.44±1.61^{b}
180.9(mg/kgbw)	15.20±1.61^{a}	10.16±1.36^{b}	6.74±1.24^{cb}	4.24±0.64^{cb}	3.84±0.74^{cb}	3.18±0.80^{cbd}	7.38±1.60^{b}
350(mg/kgbw)	14.88±2.84^{a}	10.12±0.97^{b}	7.48±1.44^{b}	5.44±2.20^{cb}	4.32±1.25^{cb}	3.12±0.70^{cd}	7.40±1.73^{b}

Results are expressed as Means ± SD for five mice per group. Values followed by the same superscript are not statistically different (P≤0.05; analysed by ANOVA followed by Tukey's post hoc test.

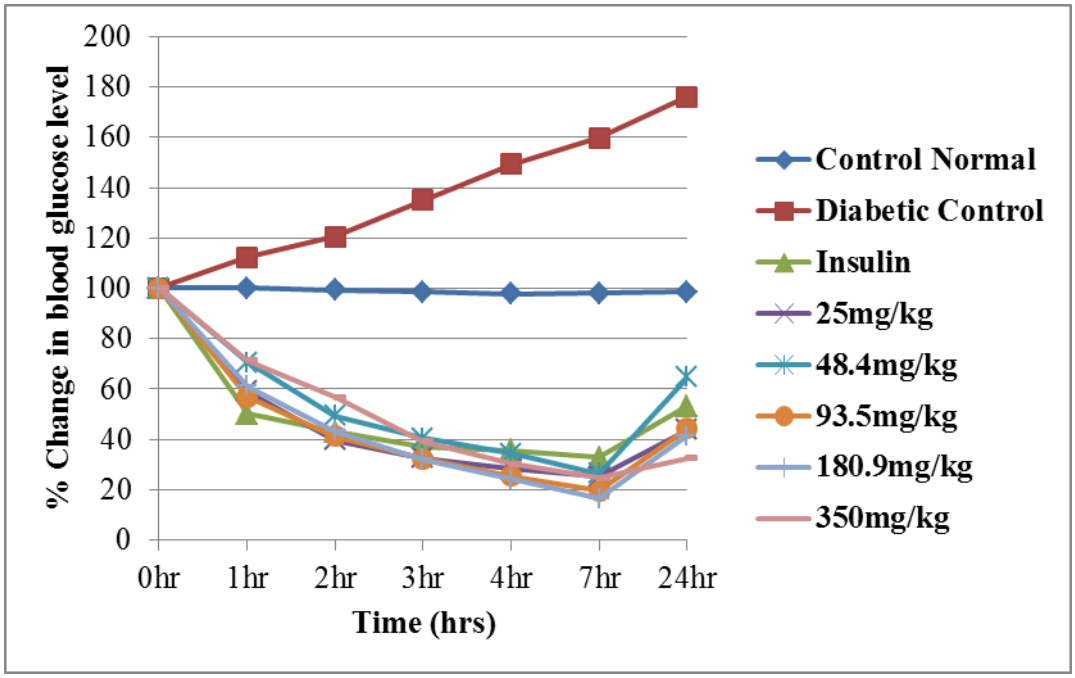

Figure 4.3: Mean percentage change in blood glucose levels of mice intraperitoneally administered with *Azadiratchta indica*

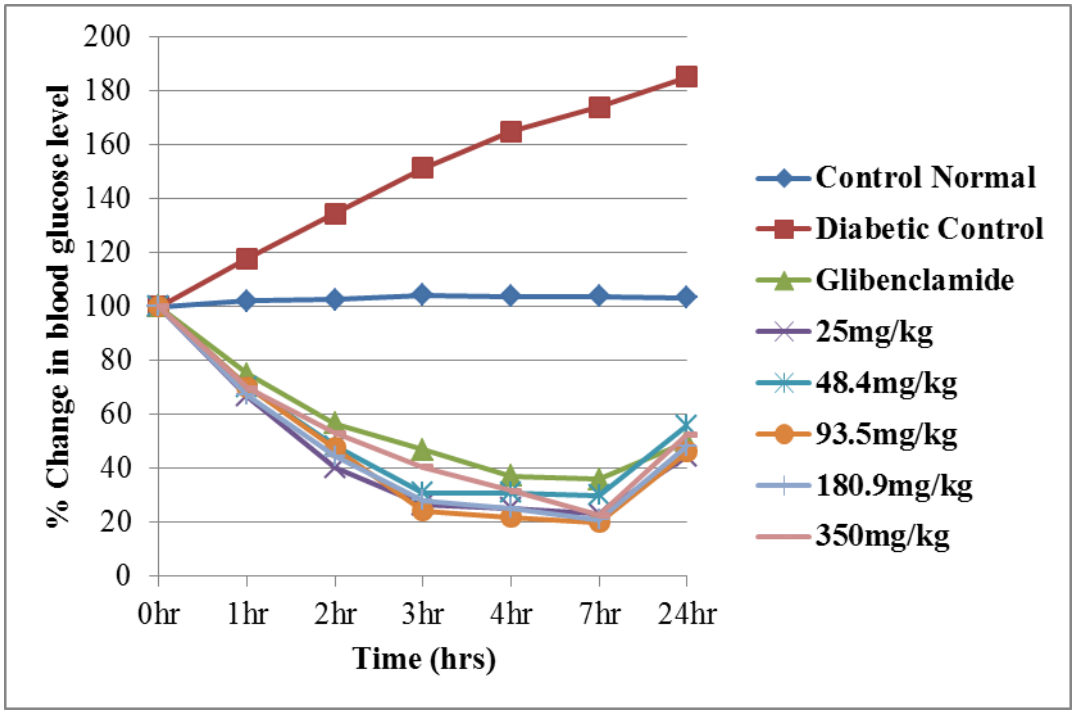

Figure 4.4: Mean percentage change in blood glucose levels of mice orally administered with *Azadiratchta indica*

4.1.3 *In vivo* hypoglycemic activity of *Lippia javanica*

The aqueous leaf extracts yielded a 9.8% (w/w) brown powder. Intraperitoneally the extract lowered the blood glucose levels at all the five doses of 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight (Table 4.5). The pattern of decrease varied with each dose upto the seventh hour. However, the sugar levels were not reduced in a dose related manner. In the first hour, the extract lowered blood glucose levels to 74.1%, 64.0%, 59.9%, 67.6% and 62.7% for 25, 48.4, 93.5, 180.9 and 350 body weight doses, respectively, compared to insulin treated diabetic mice whose blood sugar levels was lowered to 46.8% within the first hour. By the fourth hour, all the five doses (25, 48.4, 93.5, 180.9 and 350 mg/kg body weight) had lowered blood sugar levels to 42.5%, 36.0%, 36.4%, 37.1% and 37.5%, respectively, compared to insulin treated diabetic mice whose sugar levels was lowered to 37.6% within the same hour (Figure 4.5).

Orally the extract lowered blood glucose levels at all the five doses of 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight (Table 4.6), from the first hour to the twenty fourth hours in a dose-independent manner. By the second hour the extract had decreased the blood glucose levels to 66.3%, 49.3%, 48.0%, 51.8% and 50.5% respectively, for the five doses, compared to 59.2% decrease in blood sugar levels for the conventional oral drug, glibenclamide (Figure 4.6).

Table 4.5: Effects of intraperitoneally administered aqueous leaf extract of *Lippia javanica* on blood glucose levels in alloxan induced diabetic mice

	BLOOD GLUCOSE LEVELS AT VARYING TIMES (mmole/l)						
TREATMENT	**0hr**	**1hr**	**2hr**	**3hr**	**4hr**	**7hr**	**24hr**
Control/Saline	5.26±0.05 ^{b}	5.28±0.08 ^{d}	5.30±0.07 ^{c}	5.26±0.89 ^{c}	5.30±0.07 ^{b}	5.26±0.05 ^{cb}	5.28±0.08 ^{d}
Diabetic/Saline	14.28±0.93 ^{a}	15.58±1.12 ^{a}	16.64±1.13 ^{a}	18.00±0.99 ^{a}	19.08±1.20 ^{a}	21.00±1.87 ^{a}	23.30±1.61 ^{a}
Diabetic/insulin	14.66±1.44 ^{a}	6.84±0.42 ^{cd}	6.10±0.14 ^{c}	5.78±0.15 ^{cb}	5.46±0.26 ^{b}	5.04±0.15 ^{cb}	8.12±0.54 ^{c}
25(mg/kgbw)	15.24±2.90 ^{a}	11.44±3.31 ^{b}	8.58±2.01 ^{b}	7.28±1.59 ^{b}	6.44±1.15 ^{b}	6.04±0.96 ^{b}	10.86±1.62 ^{b}
48.4(mg/kgbw)	14.88±2.65 ^{a}	9.56±2.03 ^{cb}	7.26±1.38 ^{cb}	6.00±0.90 ^{cb}	5.28±0.50 ^{b}	4.68±0.31 ^{cb}	8.88±2.28 ^{cb}
93.5(mg/kgbw)	14.74±1.73 ^{a}	8.78±0.95 ^{cbd}	7.12±0.68 ^{cb}	5.90±1.05 ^{cb}	5.34±0.82 ^{b}	4.76±0.15 ^{cb}	8.66±0.70 ^{cb}
180.9(mg/kgbw)	14.36±3.28 ^{a}	9.76±2.77 ^{cb}	7.60±1.88 ^{cb}	5.94±1.38 ^{cb}	5.24±0.72 ^{b}	4.60±0.68 ^{cb}	9.08±1.17 ^{cb}
350(mg/kgbw)	13.36±0.80 ^{a}	8.36±0.61 ^{cbd}	6.46±0.51 ^{cb}	5.50±0.44 ^{cb}	5.00±0.14 ^{b}	4.24±0.23 ^{c}	6.88±0.27 ^{cd}

Results are expressed as Means ± SD for five mice per group. Values followed by the same superscript are not statistically different (P≤0.05; analysed by ANOVA followed by Tukey's post hoc test.

Table 4.6: Effects of orally administered aqueous leaf extract of *Lippia javanica* on blood glucose levels in alloxan induced diabetic mice

	BLOOD GLUCOSE LEVELS AT VARYING TIMES (mmole/l)						
TREATMENT	**0hr**	**1hr**	**2hr**	**3hr**	**4hr**	**7hr**	**24hr**
Control/Saline	5.34±0.11 ^{b}	5.34±0.13 ^{c}	5.36±0.05^{c}	5.34±0.11^{c}	5.34±0.15^{b}	5.32±0.08^{cb}	5.36±0.09 ^{e}
Diabetic/Saline	14.26±1.43^{a}	15.50±1.49 ^{a}	16.36±1.38^{a}	17.48±1.50^{a}	18.80±1.47^{a}	20.26±1.07^{a}	23.56±0.87 ^{a}
Diabetic/ Glen	14.14±1.42 ^{a}	10.78±1.17^{b}	8.38±0.90^{cb}	6.82±0.52^{cb}	5.60±0.46 ^{b}	5.14±0.17^{cb}	9.32±0.71 ^{cbd}
25(mg/kgbw)	15.36±2.82 ^{a}	12.68±3.27^{ba}	10.38±3.32^{b}	7.50±1.91^{b}	6.06±0.89 ^{b}	5.52±0.54^{b}	12.28±2.46 ^{b}
48.4(mg/kgbw)	14.92±4.69 ^{a}	10.38±2.81^{b}	7.12±1.52^{cb}	5.36±0.61^{cb}	4.84±0.49 ^{b}	4.60±0.41^{cb}	10.12±2.71^{cb}
93.5(mg/kgbw)	14.16±1.98 ^{a}	8.90±2.15^{bc}	6.88±2.23^{cb}	5.34±0.83^{c}	5.02±0.82 ^{b}	4.38±0.65^{c}	9.32±0.61 ^{cbd}
180.9(mg/kgbw)	14.00±2.50 ^{a}	9.88±2.41^{b}	7.26±1.45^{cb}	5.78±1.00^{cb}	5.22±0.92 ^{b}	4.54±0.60^{cb}	8.90±1.53 ^{cd}
350(mg/kgbw)	14.18±1.02 ^{a}	9.08±1.04^{bc}	7.16±0.65^{cb}	5.72±0.79cb	5.02±0.30 ^{b}	4.20±0.23^{c}	6.74±0.43^{ed}

Results are expressed as Means ± SD for five mice per group. Values followed by the same superscript are not statistically different (P≤0.05; analysed by ANOVA followed by Tukey's post hoc test.

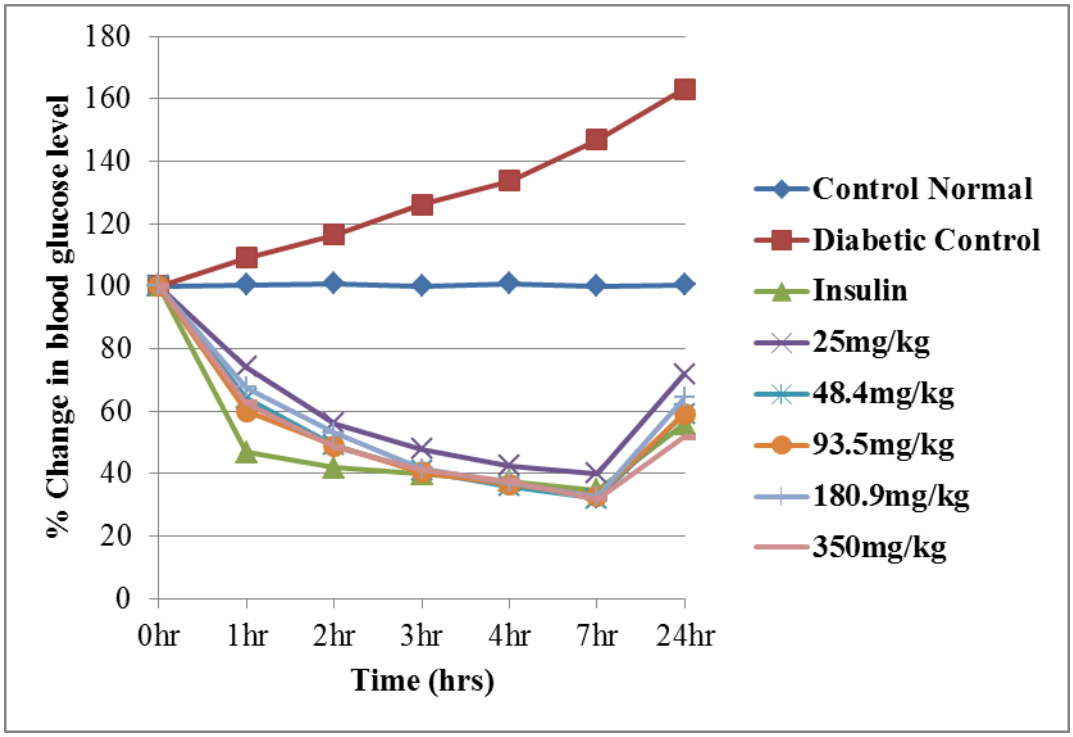

Figure 4.5: Mean percentage change in blood glucose levels of mice intraperitoneally administered with *Lippia javanica*

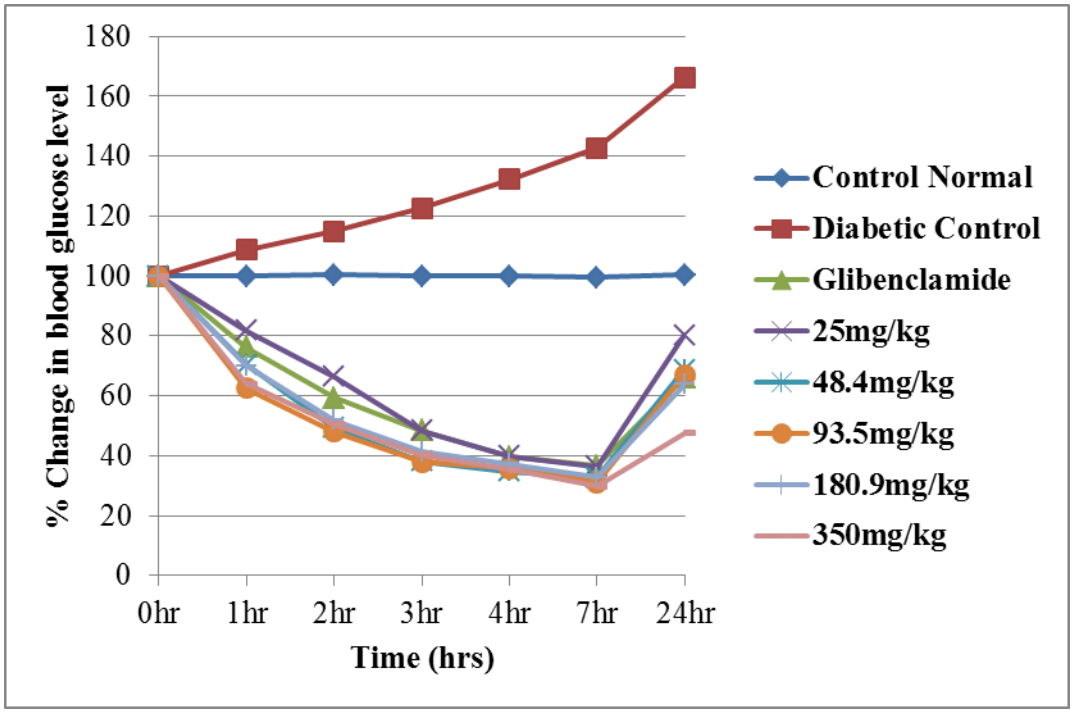

Figure 4.6:Mean percentage change in blood glucose levels of mice orally administered with *Lippia javanica*

4.1.4 *In vivo* hypoglycemic activity of *Ocimum lamiifolium*

A leaf extract yielded a 8% (w/w) light brown powder. Intraperitoneally administered extract lowered the blood glucose levels at all the five doses of 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight (Table 4.7). This occurred in two phases, in the first one hour the extract caused a steep decline in blood glucose levels, followed by a steady decline upto to the seventh hour. After this, a gradual increase was recorded in the twenty fourth hour. However, the reduction in sugar level was not in a dose related manner during the two phases.

In the first hour, the extract lowered blood glucose levels to 67.8%, 59.5%, 54.4%, 65.7% and 53.9% for 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight doses, respectively, compared to insulin treated diabetic mice whose blood sugar levels was lowered to 48.4% within the first hour. By the fourth hour, all the five doses (25, 48.4, 93.5, 180.9 and 350 mg/kg body weight) had lowered blood sugar levels to 37.8%, 27.0%, 27.5%, 37.2% and 26.2%, respectively, compared to insulin treated diabetic mice whose sugar levels was lowered to 36.3% within the same hour (Figure 4.7).

Orally the extract lowered blood glucose levels at all the five doses of 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight (Table 4.8), from the first hour to the twenty fourth hours in a dose independent manner. By the second hour the extract had lowered the blood glucose levels to 60.0%, 34.8%, 30.0%, 40.4% and 38.1%, respectively for the five doses, compared to 61.5% for the conventional oral drug, glibenclamide.

Table 4.7: Effects of intraperitoneally administered aqueous leaf extract of *Ocimum lamifoliium* on blood glucose levels in alloxan induced diabetic mice

	BLOOD GLUCOSE LEVELS AT VARYING TIMES (mmole/l)						
TREATMENT	**0hr**	**1hr**	**2hr**	**3hr**	**4hr**	**7hr**	**24hr**
Control/Saline	5.28±0.19 [b]	5.26±0.15 [b]	5.26±0.09 [b]	5.34±0.09 [b]	5.26±0.09 [b]	5.28±0.13 [b]	5.22±0.08 [c]
Diabetic/Saline	15.18±4.65 [a]	16.46±4.63 [a]	17.74±4.27 [a]	19.40±4.42 [a]	20.58±4.46 [a]	21.92±4.41 [a]	25.26±3.67 [a]
Diabetic/insulin	15.60±4.95 [a]	7.06±0.21 [b]	6.20±0.25 [b]	5.64±0.47 [b]	5.20±0.41 [b]	4.94±0.28 [b]	7.46±0.65 [cb]
25(mg/kgbw)	16.82±4.53 [a]	11.78±5.66 [ba]	9.28±3.10 [b]	7.58±2.11 [b]	6.26±1.24 [b]	5.46±1.01 [b]	11.64±2.90 [b]
48.4(mg/kgbw)	15.18±2.81 [a]	9.16±3.27 [b]	5.60±0.74 [b]	4.72±0.49 [b]	4.00±0.58 [b]	3.40±0.49 [b]	7.56±1.39 [cb]
93.5(mg/kgbw)	15.32±3.06 [a]	8.30±1.59 [b]	5.72±0.80 [b]	4.68±0.54 [b]	4.16±0.65 [b]	3.46±0.69 [b]	8.48±2.59 [cb]
180.9(mg/kgbw)	15.90±5.49 [a]	10.60±4.66 [ba]	7.92±2.63 [b]	6.38±1.89 [b]	5.70±1.13 [b]	5.12±0.93 [b]	9.88±2.33 [b]
350(mg/kgbw)	17.94±3.42 [a]	9.58±1.30 [ba]	6.62±0.61 [b]	5.38±0.70 [b]	4.62±0.71 [b]	4.04±0.84 [b]	8.04±1.04 [cb]

Results are expressed as Means ± SD for five mice per group. Values followed by the same superscript are not statistically different (P≤0.05; analysed by ANOVA followed by Tukey's post hoc test.

Table 4.8: Effects of orally administered aqueous leaf extract of *Ocimum lamifoliium* on blood glucose levels in alloxan induced diabetic mice

	BLOOD GLUCOSE LEVELS AT VARYING TIMES (mmole/l)						
TREATMENT	**0hr**	**1hr**	**2hr**	**3hr**	**4hr**	**7hr**	**24hr**
Control/Saline	5.22±0.08 [b]	5.26±0.05[e]	5.22±0.08[ced]	5.18±0.08[cd]	5.24±0.05[cbd]	5.28±0.08[cb]	5.10±0.10[c]
Diabetic/Saline	13.52±2.22 [a]	14.86±1.80 [a]	16.04±1.89[a]	17.36±1.61[a]	18.74±1.84[a]	20.56±2.10[a]	22.56±2.72[a]
Diabetic/ Glen	15.56±2.78 [a]	12.90±2.47[ba]	9.62±2.55[b]	7.80±1.57[b]	6.18±1.25[cb]	5.38±0.60[cb]	8.22±0.86 [b]
25(mg/kgbw)	13.66±2.46 [a]	9.86±1.62[bcd]	9.86±1.62[b]	7.56±0.50[b]	7.56±0.50[b]	6.72±0.30[b]	5.92±0.68[c]
48.4(mg/kgbw)	13.52±2.36 [a]	9.06±1.95[cd]	4.70±1.58[ed]	4.42±1.11[cd]	3.78±0.81[d]	3.74±1.01[c]	8.00±1.47[cb]
93.5(mg/kgbw)	12.60±1.04 [a]	6.84±0.94[ed]	3.68±1.21e	3.44±1.08[d]	3.28±1.16[d]	3.48±1.08[c]	7.66±2.01[cb]
180.9(mg/kgbw)	17.38±3.52 [a]	11.32±1.88[bc]	6.94±0.96[cbd]	6.36±0.86[cb]	5.28±0.54[cbd]	4.92±0.22[cb]	8.26±1.08 [b]
350(mg/kgbw)	14.32±2.47 [a]	8.52±1.70[ecd]	5.50±1.97[ced]	4.88±1.32[cd]	4.20±0.93[cd]	3.70±0.96[c]	5.12±0.66[c]

Results are expressed as Means ± SD for five mice per group. Values followed by the same superscript are not statistically different (P≤0.05; analysed by ANOVA followed by Tukey's post hoc test.

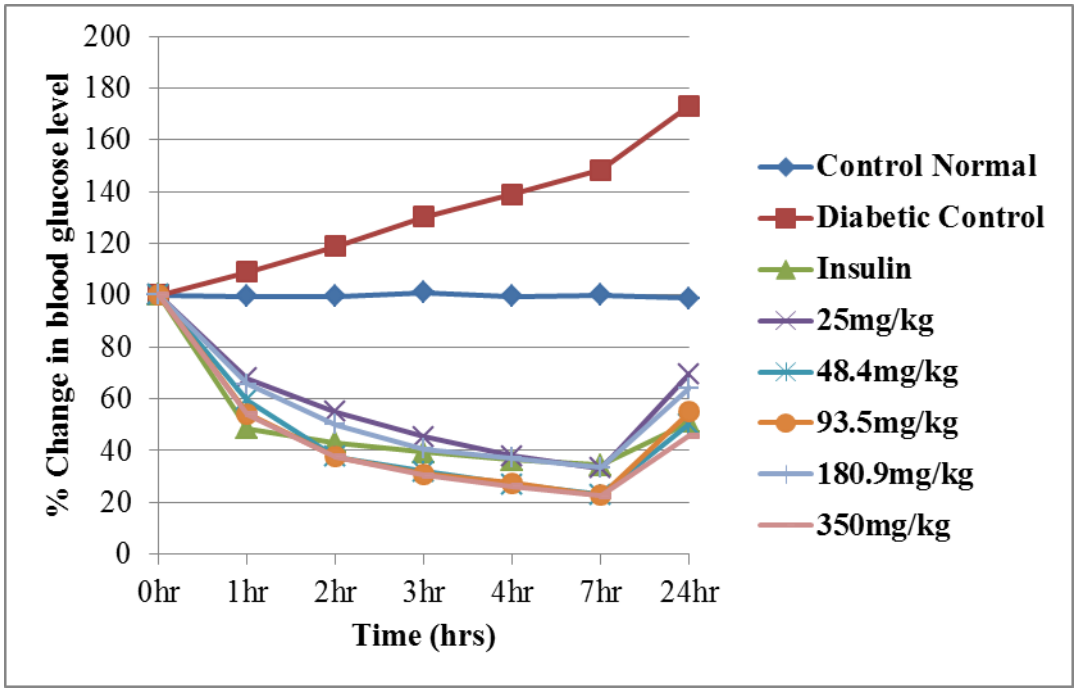

Figure 4.7: Mean percentage change in blood glucose levels of mice intraperitoneally administered with *Ocimum lamiifolium*

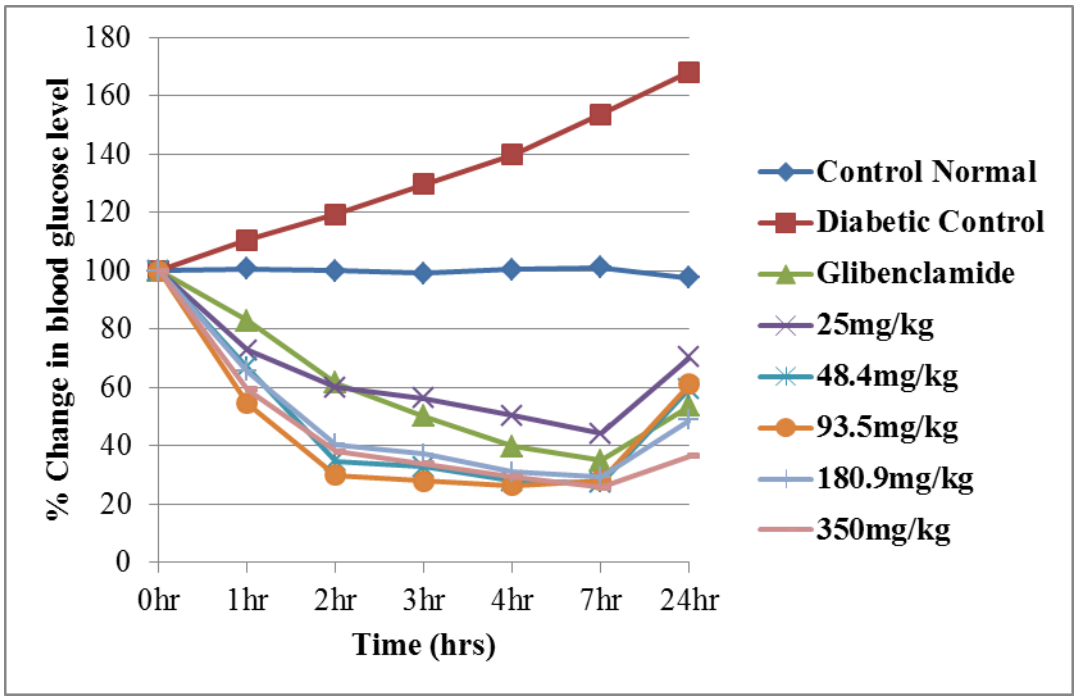

Figure 4.8: Mean percentage change in blood glucose levels of mice orally administered with *Ocimum lamiifolium*

4.1.5 *In vivo* hypoglycemic activity of *Persea americana*

The Leaf extract yielded yielded 8% (w/w) dark brown granules. Intraperitoneally administrered extract lowered the blood glucose levels at all the five doses of 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight (Table 4.9). The plant extract caused a steady decline in blood glucose levels from the first to the seventh hour. The blood sugar levels reduced in a dose-independent manner. In the first hour, the extract lowered blood glucose levels to 74.1%, 64.0%, 59.9%, 67.6%, and 62.7% for 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight doses, respectively, compared to insulin treated diabetic mice whose blood sugar levels was lowered to 46.8% within the first hour. By the third hour, all the five doses (25, 48.4, 93.5, 180.9 and 350 mg/kg body weight) had lowered blood sugar levels to 47.7%, 40.8%, 40.5%, 41.4% and 41.2%, respectively, compared to insulin treated diabetic mice whose sugar levels was lowered to 39.8% within the same hour (Figure 4.9).

Orally the extract lowered blood glucose levels at 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight doses (Table 4.10), steadly from the first hour to the seventh hour in dose independent manner. By the second hour the extract had lowered the blood glucose levels to 51.7%, 58.9%, 58.9%, 56.8% and 59.1%, respectively for the five doses, compared to 57.8% for the conventional oral drug, glibenclamide (Figure 4.8).

Table 4.9: Effects of intraperitoneally administered aqueous leaf extract of *Persea americana* on blood glucose levels in alloxan induced diabetic mice

	BLOOD GLUCOSE LEVELS AT VARYING TIMES (mmole/l)						
TREATMENT	**0hr**	**1hr**	**2hr**	**3hr**	**4hr**	**7hr**	**24hr**
Control/Saline	5.14±0.11 ^{b}	5.14±0.13 ^{d}	5.16±0.05 ^{c}	5.14±0.11 ^{c}	5.14±0.15 ^{c}	5.12±0.08 ^{cb}	5.16±0.09 ^{d}
Diabetic/Saline	12.84±1.51^{a}	13.90±1.32 ^{a}	14.76±1.42 ^{a}	15.84±1.36 ^{a}	17.28±1.12 ^{a}	18.66±1.43 ^{a}	22.32±0.88 ^{a}
Diabetic/insulin	13.70±1.34 ^{a}	7.42±0.53 ^{dc}	6.52±0.41 ^{c}	5.74±0.44 ^{c}	5.14±0.15 ^{c}	4.72±0.28 ^{cb}	8.78±0.54 ^{c}
25(mg/kgbw)	15.92±2.38 ^{a}	13.12±2.48 ^{a}	9.90±1.69 ^{b}	7.94±1.19 ^{b}	6.46±0.51 ^{b}	5.70±0.63 ^{b}	11.80±1.66 ^{b}
48.4(mg/kgbw)	14.38±2.87 ^{a}	10.22±2.46 ^{ac}	7.76±1.41 ^{cb}	5.66±0.59 ^{c}	5.06±0.30 ^{c}	4.64±0.34 ^{cb}	10.20±2.08 ^{cb}
93.5(mg/kgbw)	15.02±2.15 ^{a}	9.86±2.25 ^{c}	7.74±2.01 ^{cb}	5.68±0.97 ^{c}	5.00±0.79 ^{c}	4.26±0.58 ^{c}	9.30±0.68 ^{c}
180.9(mg/kgbw)	14.98±2.55 ^{a}	10.84±2.31 ^{ac}	7.58±1.79 ^{cb}	5.96±0.97 ^{c}	5.16±0.38 ^{c}	4.80±0.38 ^{cb}	10.18±1.54 ^{cb}
350(mg/kgbw)	14.64±2.30 ^{a}	10.14±1.94 ^{ac}	7.68±1.33 ^{cb}	6.02±0.97 ^{c}	4.94±0.72 ^{c}	4.04±0.53 ^{c}	6.28±0.88 ^{d}

Results are expressed as Means ± SD for five mice per group. Values followed by the same superscript are not statistically different (P≤0.05; analysed by ANOVA followed by Tukey's post hoc test.

Table 4.10: Effects of orally administered aqueous leaf extract of *Persea americana* on blood glucose levels in alloxan induced diabetic mice

	BLOOD GLUCOSE LEVELS AT VARYING TIMES (mmole/l)						
TREATMENT	**0hr**	**1hr**	**2hr**	**3hr**	**4hr**	**7hr**	**24hr**
Control/Saline	5.12±0.08^{c}	5.20±0.07^{c}	5.22±0.04^{c}	5.16±0.09 ^{b}	5.14±0.09 ^{b}	5.12±0.08 ^{b}	5.14±0.11^{d}
Diabetic/Saline	16.30±3.04^{ba}	17.74±3.34^{a}	19.26±3.76^{a}	21.30±3.46^{a}	23.52±3.71^{a}	24.42±3.89^{a}	26.74±3.28^{a}
Diabetic/ Glen	15.04±1.92^{ba}	11.22±2.78^{b}	8.82±2.75^{cb}	7.20±1.76 ^{b}	6.20±1.19 ^{b}	5.26±0.52 ^{b}	7.52±0.63^{cbd}
25(mg/kgbw)	18.76±3.69^{a}	11.46±2.76^{b}	9.76±2.84^{b}	7.04±1.45 ^{b}	6.20±0.79 ^{b}	5.76±0.59 ^{b}	11.12±2.34^{b}
48.4(mg/kgbw)	14.34±1.82^{b}	10.54±1.43^{b}	8.42±0.95^{cb}	7.42±0.60 ^{b}	6.40±0.83 ^{b}	5.78±0.70 ^{b}	9.92±1.14^{cb}
93.5(mg/kgbw)	13.30±1.57^{b}	8.60±1.17^{cb}	7.80±1.02^{cb}	6.72±0.37 ^{b}	6.00±0.35 ^{b}	5.00±0.41 ^{b}	9.00±1.19^{cb}
180.9(mg/kgbw)	13.46±1.76^{b}	8.60±1.46^{cb}	7.66±1.12^{cb}	6.34±0.48 ^{b}	5.56±0.51 ^{b}	4.96±0.11 ^{b}	8.70±1.62^{cbd}
350(mg/kgbw)	12.80±0.98^{b}	9.58±1.56^{b}	7.56±0.98^{cb}	6.26±0.64 ^{b}	4.92±0.50 ^{b}	4.50±0.67 ^{b}	6.94±1.71^{cd}

Results are expressed as Means ± SD for five mice per group. Values followed by the same superscript are not statistically different (P≤0.05; analysed by ANOVA followed by Tukey's post hoc test.

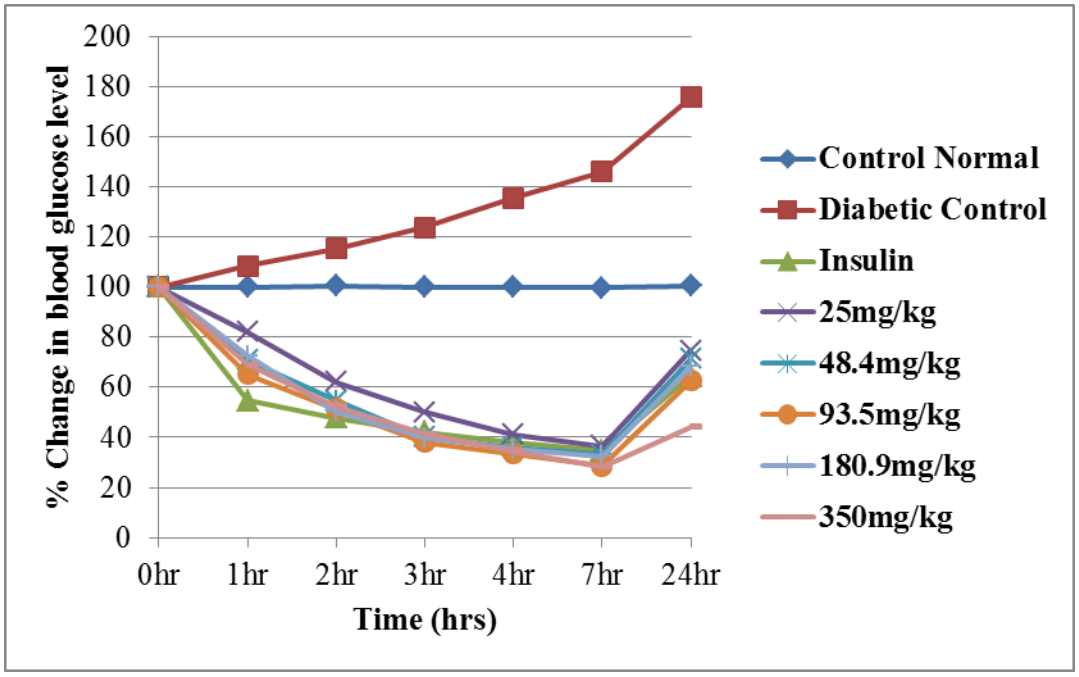

Figure 4.9: Mean percentage change in blood glucose levels of mice intraperitoneally administered with *Persea americana*

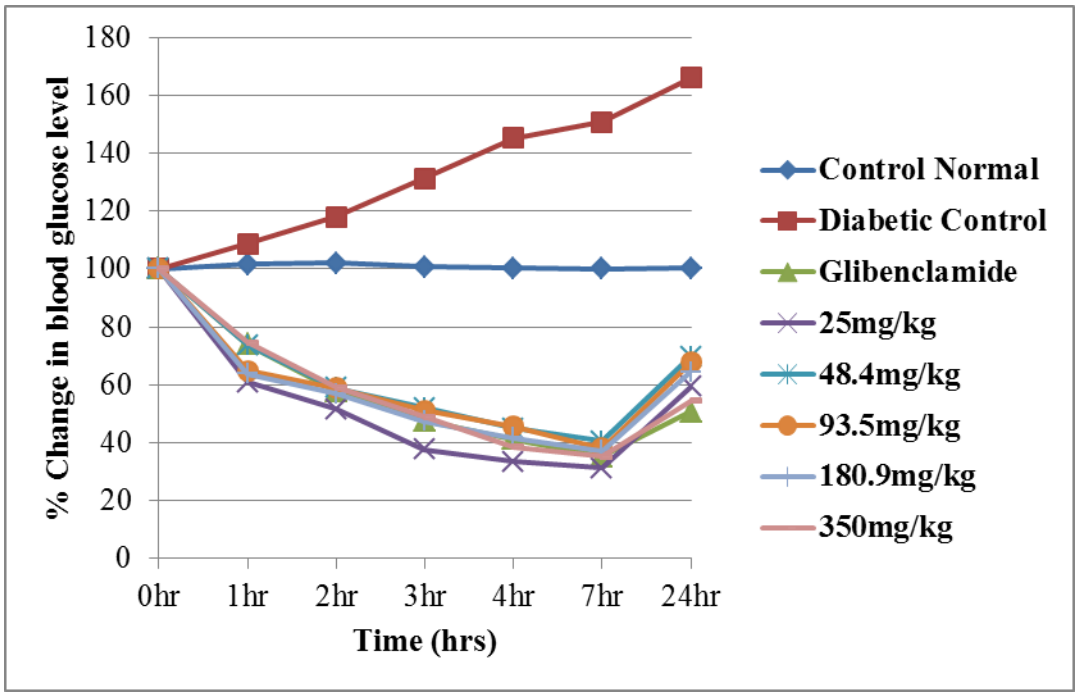

Figure 4.10: Mean percentage change in blood glucose levels of mice orally administered with *Persea americana*

4.1.6 A comparison of antidiabetic effect of intraperitoneal and oral administration of the five aqueous plant extracts in mice models

Tables 11 and 12 shows the hypoglycemic effect of intraperitoneal and oral administration of 25mg/kg body weight of different plant extracts in mice. Results show that in both routes, the optimal dose for all the extracts occurred between the third and the seventh hour where the sugar reduction rate was between 44% and 77%. In the third hour, the percent reductions in blood glucose (intraperitoneally) were at 44.14% for *C. macrostachyus,* 32.56% for *A. indica*, 47.73% for *L. javanica,* 45.21% for *O. lamiifolium* and 50.20% for *P. americana* (Figure 4.11). By the seventh hour, the blood sugar levels had been reduced to 30.32% for *C. macrostachyus,* 25.26% for *A. indica,* 40% for *L. javanica,* 33.17% for *O. lamiifolium* and 36.57% for *P. americana* (Figure 4.11).

The oral administration of the extracts at the same dose lowered blood glucose levels appreciably from the first hour to the seventh hour. By the third hour the extracts had lowered the blood glucose levels to 52.61% for *C. macrostachyus*, 26.52% of *A. indica,* 48.40% for *L. javanica*, 56.42% for *O. lamiifolium* and 37.65% for *P. americana* (Figure 4.12). In the seventh hour, the percentage reductions were at 39.83% for *C. macrostachyus,* 23.13% for *A. indica,* 36.48% for *L. javanica,* 44.23% for *O. lamiifolium* and 31.19% for *P. americana* (Figure 4.12). *A. indica* demonstrated the highest percentage reduction in blood sugar levels at this dose. In the twenty fourth hour the mice had returned to diabetic states as by all the extracts.

Table 4.11: Antidiabetic effect of intraperitoneal administration of the five aqueous plant extracts in mice at 25mg/kg body weight dose

Treatment	Plant Extracts	Time (hrs)						
		0hr	1hr	2hr	3hr	4hr	7hr	24hr
25mg/kgbwt	*Croton macrostachyus*	15.42±1.26^{a}	12.68±3.11^{a}	8.90±1.64^{a}	6.80±1.06^{a}	5.44±0.78^{a}	4.68±0.48^{ab}	6.64±1.15^{c}
25mg/kgbwt	*Azardiratchta indica*	17.84±3.78^{a}	10.32±1.59^{a}	7.00±1.33^{a}	5.70±0.97^{a}	4.94±0.75^{a}	4.40±0.58^{b}	7.52±0.73^{bc}
25mg/kgbwt	*Lippia javanica*	15.24±2.90^{a}	11.44±3.31^{a}	8.58±2.02^{a}	7.28±1.59^{a}	6.44±1.15^{a}	6.04±0.96^{a}	10.86±1.62^{ab}
25mg/kgbwt	*Ocimum lamiifolium*	16.82±4.53^{a}	11.78±5.66^{a}	9.28±3.10^{a}	7.58±2.11^{a}	6.26±1.25^{a}	5.46±1.01^{ab}	11.64±2.90^{a}
25mg/kgbwt	*Persea americana*	15.92±2.38^{a}	13.12±2.48^{a}	9.90±1.69^{a}	7.94±1.19^{a}	6.46±0.51^{a}	5.70±0.63^{ab}	11.80±1.66^{a}

Results are expressed as Means ± SD for five mice per group. Values followed by the same superscript are not statistically different ($p \leq 0.05$); analysed by ANOVA followed by Tukey's post hoc test.

Table 4.12: Antidiabetic effect of oral administration of the five aqueous plant extracts in mice at 25mg/kg body weight dose

Treatment	Plant Extracts	Time (hrs)						
		0hr	1hr	2hr	3hr	4hr	7hr	24hr
25mg/kgbwt	*Croton macrostachyus*	12.96±3.59^{b}	12.44±4.84^{a}	9.14±2.37^{ab}	6.52±1.45^{a}	5.02±0.88^{bc}	4.80±0.86^{a}	10.04±1.62^{a}
25mg/kgbwt	*Azardiratchta indica*	14.56±0.57^{ab}	9.68±1.20^{a}	5.86±0.79^{b}	3.88±0.80^{b}	3.70±0.46^{c}	3.36±0.40^{b}	6.44±0.99^{b}
25mg/kgbwt	*Lippia javanica*	15.36±2.82^{ab}	12.68±3.27^{a}	10.38±3.33^{a}	7.50±1.91^{a}	6.06±0.89^{ab}	5.52±0.54^{a}	12.28±2.46^{a}
25mg/kgbwt	*Ocimum lamiifolium*	13.66±2.46^{ab}	9.86±1.62^{a}	8.04±0.57^{ab}	7.56±0.50^{a}	6.72±0.30^{a}	5.92±0.68^{a}	9.30±1.07^{ab}
25mg/kgbwt	*Persea Americana*	18.76±3.69^{a}	11.46±2.76^{a}	9.76±2.84^{ab}	7.04±1.45^{a}	6.20±0.79^{ab}	5.76±0.59^{a}	11.12±2.34^{a}

Results are expressed as Means ± SD for five mice per group. Values followed by the same superscript are not statistically different ($p \leq 0.05$); analysed by ANOVA followed by Tukey's post hoc test.

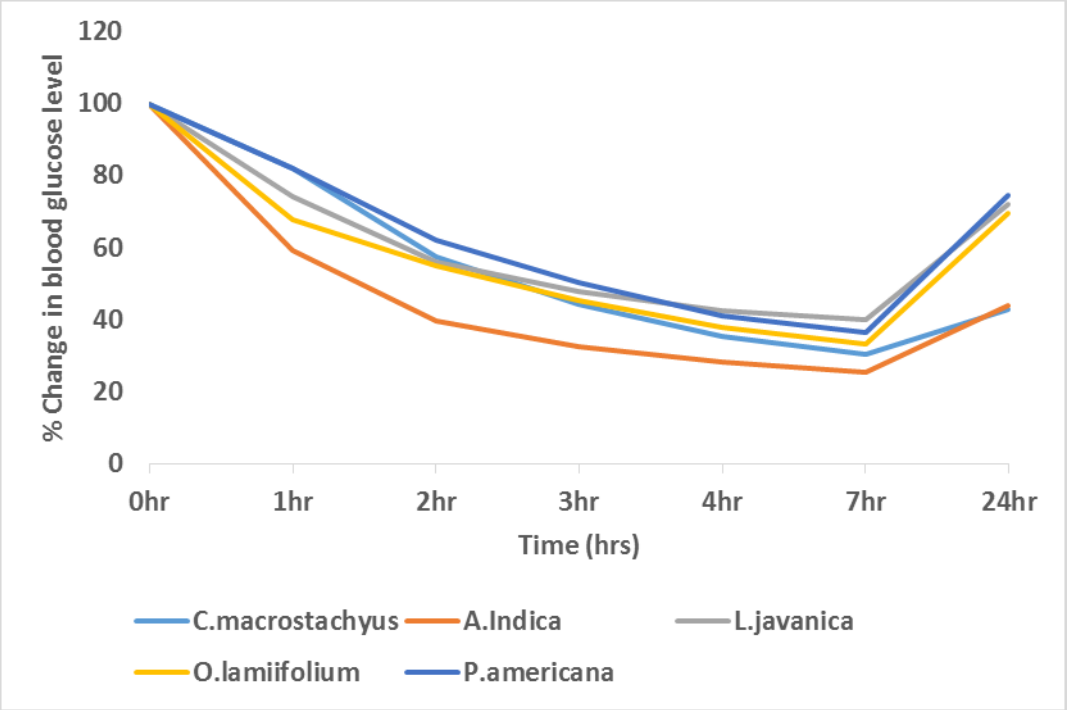

Figure 4.11: A comparison of mean percentage change in blood glucose levels of mice intraperitoneally administered with extracts at 25mg/kg body weight

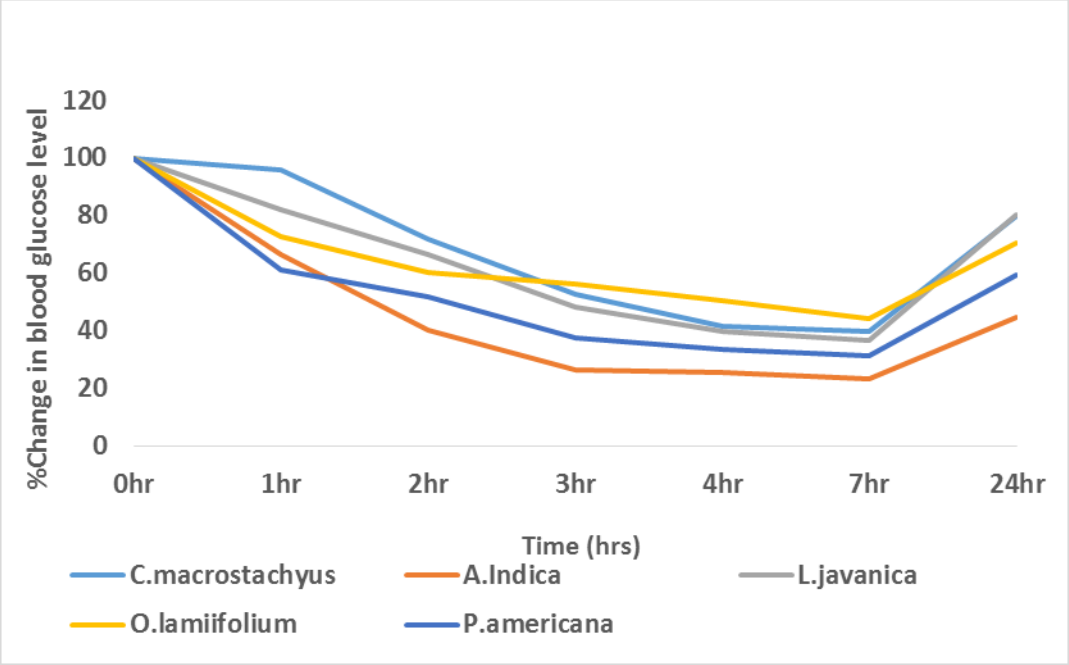

Figure 4.12: A comparison of mean percentage change in blood glucose levels of mice orally administered with extracts at 25mg/kg body weight

Tables 13 and 14 shows the hypoglycemic effect of intraperitoneal and oral administration of 48.4mg/kg body weight of different plant extracts in mice. Results show that in both routes, the optimal dose for all the extracts occurred between the third and the seventh hour where the sugar reduction rate was between 45% and 78%. In the third hour, the intraperitoneal administration of aqueous leaf extracts of five plants lowered the blood glucose levels to 46.41% for *C. macrostachyus,* 40.53% for *A. indica,* 40.79% for *L. javanica,* 31.82% for *O.lamiifolium* and 40.02% for *P. americana* (Figure 4.13). By the seventh hour, the blood sugar levels had been reduced to 31.64% for *C. macrostachyus,* 26.49% for *A. indica,* 31.99% for *L. javanica,* 22.91% for *O. lamiifolium* and 33.19% for *P. americana* (Figure 4.13). As the figure shows, *O. lamiifolium* and *A. indica* lowered the blood sugar level by a higher percentage than other plants and these values remained persistent upto the seventh hour.

In the third hour, the percentage reductions in blood glucose level following an oral administration of the extracts were at 55.46% for *C. macrostachyus*, 30.99% for *A. indica*, 38.05% for *L. javanica,* 32.71% for *O.lamiifolium* and 52.07% for *P. Americana* (Figure 4.14). By the seventh hour *C. macrostachyus, A. indica, L. javanica, O. lamiifolium* and *P. americana* had lowered the blood glucose levels to 41.38%, 29.94%, 32.87%, 27.30% and 40.48% respectively (Figure 4.14). However, in the twenty fourth hour, blood glucose levels in both routes had risen but the percentage change were insignificant ($p \leq 0.05$) (Tables 4.12-13).

Table 4.13: Antidiabetic effect of intraperitoneal administration of the five aqueous plant extracts in mice at 48.4mg/kg body weight dose

Treatment	Plant Extracts	Time (hrs)						
		0hr	1hr	2hr	3hr	4hr	7hr	24hr
48.4mg/kgbwt	*Croton macrostachyus*	16.48±2.91^{a}	14.98±3.49^{a}	10.46±2.36^{a}	7.60±1.26^{a}	6.24±1.23^{a}	5.18±0.74^{a}	8.48±1.46^{a}
48.4mg/kgbwt	*Azardiratchta indica*	13.42±3.00^{a}	9.50±2.33^{b}	6.60±1.1^{ab}	5.38±0.86^{b}	4.54±0.62^{b}	3.50±0.51^{b}	8.32±1.02^{a}
48.4mg/kgbwt	*Lippia javanica*	14.88±2.64^{a}	9.56±2.04^{b}	7.26±1.38^{b}	6.00±0.90^{ab}	5.28±0.50^{ab}	4.68±0.31^{a}	8.88±2.28^{a}
48.4mg/kgbwt	*Ocimum lamiifolium*	15.18±2.81^{a}	9.16±3.27^{b}	5.60±0.74^{b}	4.72±0.49^{b}	4.00±0.58^{b}	3.40±0.49^{b}	7.56±1.39^{a}
48.4mg/kgbwt	*Persea americana*	14.38±2.87^{a}	10.22±2.46^{ab}	7.76±1.41^{ab}	5.66±0.60^{b}	5.06±0.31^{ab}	4.64±0.34^{a}	10.20±2.08^{a}

Results are expressed as Means ± SD for five mice per group. Values followed by the same superscript are not statistically different ($p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test.

Table 4.14: Antidiabetic effect of oral administration of the five aqueous plant extracts in mice at 48.4mg/kg body weight dose

Treatment	Plant Extracts	Time (hrs)						
		0hr	1hr	2hr	3hr	4hr	7hr	24hr
48.4mg/kgbwt	*Croton macrostachyus*	10.64±2.57^{a}	11.12±5.36^{a}	7.12±1.99^{ab}	5.76±1.37^{ab}	5.08±1.38^{ab}	4.44±1.67^{ab}	8.88±2.08^{a}
48.4mg/kgbwt	*Azardiratchta indica*	14.18±2.33^{a}	9.86±1.11^{a}	6.76±0.73^{ab}	4.28±0.43^{b}	4.30±0.45^{b}	4.22±0.79^{ab}	7.96±1.98^{a}
48.4mg/kgbwt	*Lippia javanica*	14.92±4.69^{a}	10.38±2.81^{a}	7.12±1.52^{ab}	5.36±0.61^{b}	4.84±0.49^{ab}	4.60±0.41^{ab}	10.12±2.70^{a}
48.4mg/kgbwt	*Ocimum lamiifolium*	13.52±2.36^{a}	9.06±1.95^{a}	4.70±1.58^{b}	4.42±1.12^{b}	3.78±0.81^{b}	3.74±1.01^{b}	8.00±1.47^{a}
48.4mg/kgbwt	*Persea Americana*	14.34±1.82^{a}	10.54±1.43^{a}	8.42±0.95^{a}	7.42±0.60^{a}	6.40±0.83^{a}	5.78±0.70^{a}	9.92±1.14^{a}

Results are expressed as Means ± SD for five mice per group. Values followed by the same superscript are not statistically different ($p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test.

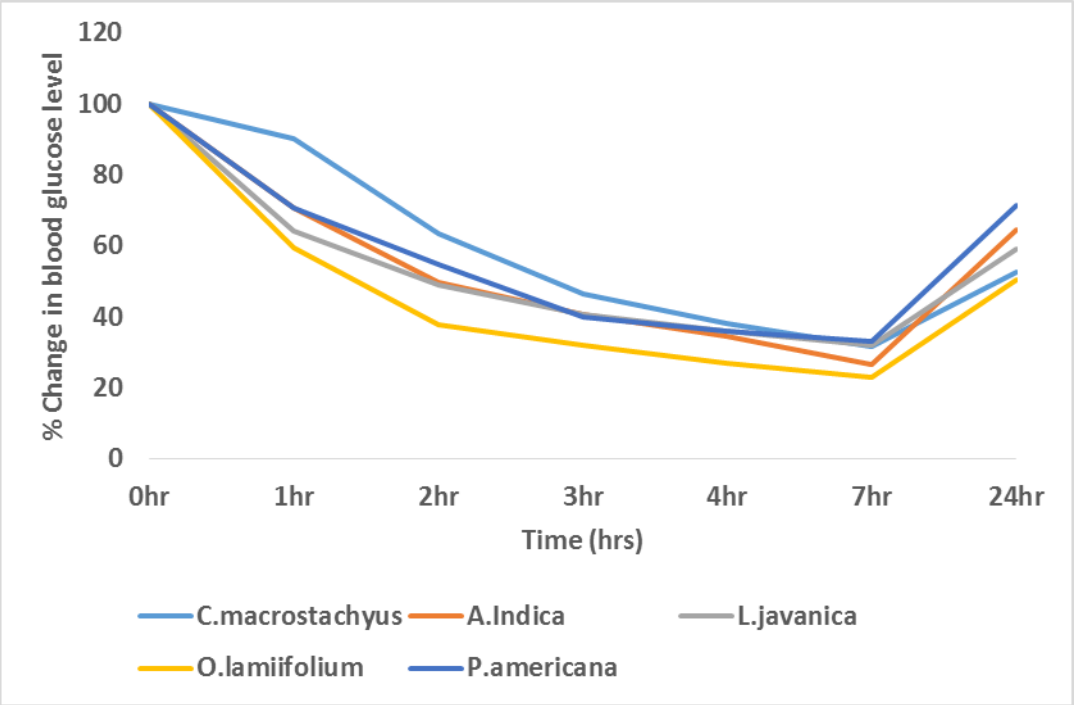

Figure 4.13: A comparison of mean percentage change in blood glucose levels of mice intraperitoneally administered with extracts at 48.4mg/kg body weight

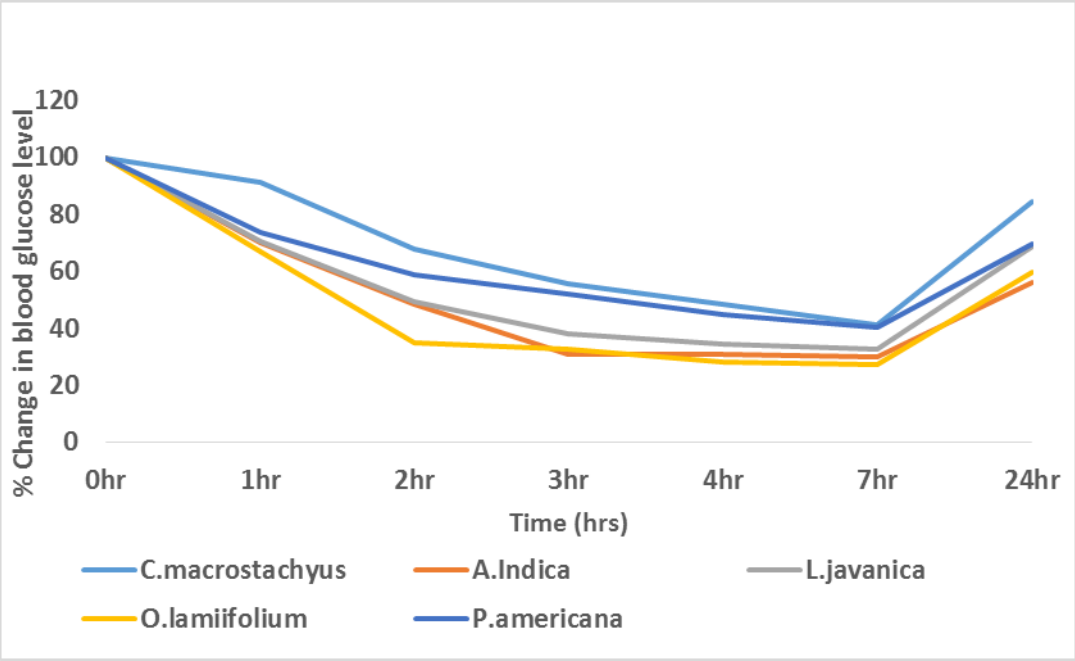

Figure 4.14: A comparison of mean percentage change in blood glucose levels of mice orally administered with extracts at 48.4mg/kg body weight

Tables 15 and 16 shows the hypoglycemic effect of intraperitoneal and oral administration of 93.5mg/kg body weight of different plant extracts in mice. Results show that in both routes, the optimal dose for all the extracts occurred between the third and the seventh hour where the sugar reduction rate was between 49% and 81%. In the third hour, the percentage reductions of the blood glucose levels were at 41.54%, 32.265, 40.50%, 31.04% and 38.17% for *C. macrostachyus, A. indica, L. javanica, O. lamiifolium and P. americana* respectively (Figure 4.15). By the seventh hour *A. indica* had recorded the highest reductions in blood glucose levels at 19.67% followed by *O. lamiifolium* at 22.73% (Figure 4.15).

The oral administration of the extracts at the same dose in mice also lowered blood glucose levels appreciably from the first hour to the seventh hour with *A. indica* and *O. lamiifolium* recording the highest reductions in blood glucose levels at 19.97% and 28.16%, respectively, by the seventh hour (Figure 4.16). In the twenty fourth hour all the plant extracts had lost their hypoglycemic activity and the mice had recovered their diabetic states (Figure 4.15-16).

Table 4.15: Antidiabetic effect of intraperitoneal administration of the five aqueous plant extracts in mice at 93.5mg/kg body weight dose

Treatment	Plant Extracts	Time (hrs)						
		0hr	1hr	2hr	3hr	4hr	7hr	24hr
93.5mg/kgbwt	*Croton macrostachyus*	13.68±2.55^{a}	7.48±0.92^{a}	6.30±0.46^{a}	5.54±0.43^{a}	4.98±0.25^{a}	4.62±0.36^{a}	8.12±1.54^{ab}
93.5mg/kgbwt	*Azardiratchta indica*	14.14±2.18^{a}	7.90±0.45^{a}	5.78±0.59^{a}	4.52±0.54^{a}	3.60±0.60^{b}	2.78±0.49^{c}	6.22±0.89^{b}
93.5mg/kgbwt	*Lippia javanica*	14.74±1.73^{a}	8.78±0.95^{a}	7.12±0.68^{a}	5.90±1.05^{a}	5.34±0.82^{a}	4.76±0.15^{a}	8.66±0.70^{ab}
93.5mg/kgbwt	*Ocimum lamiifolium*	15.32±3.06^{a}	8.30±1.60^{a}	5.72±0.80^{a}	4.68±0.54^{a}	4.16±0.65^{ab}	3.46±0.70^{bc}	8.48±2.59^{ab}
93.5mg/kgbwt	*Persea americana*	15.02±2.15^{a}	9.86±2.25^{a}	7.74±2.01^{a}	5.68±0.97^{a}	5.00±0.79^{a}	4.26±0.58^{ab}	9.30±0.68^{a}

Results are expressed as Means ± SD for five mice per group. Values followed by the same superscript are not statistically different ($p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test.

Table 4.16: Antidiabetic effect of oral administration of the five aqueous plant extracts in mice at 93.5mg/kg body weight dose

Treatment	Plant Extracts	Time (hrs)						
		0hr	1hr	2hr	3hr	4hr	7hr	24hr
93.5mg/kgbwt	*Croton macrostachyus*	12.42±1.18^{a}	8.18±1.11^{ab}	7.32±0.75^{a}	6.24±0.65^{a}	5.90±0.72^{a}	5.98±1.35^{a}	9.64±1.17^{a}
93.5mg/kgbwt	*Azardiratchta indica*	13.94±0.74^{a}	9.78±1.03^{a}	6.68±1.55^{a}	3.40±0.79^{b}	3.06±0.86^{b}	2.80±0.75^{c}	6.44±1.61^{b}
93.5mg/kgbwt	*Lippia javanica*	14.16±1.98^{a}	8.90±2.15^{ab}	6.88±2.23^{a}	5.34±0.83^{a}	5.02±0.82^{a}	4.38±0.65^{abc}	9.32±0.61^{a}
93.5mg/kgbwt	*Ocimum lamiifolium*	12.60±1.04^{a}	6.84±0.94^{b}	3.68±1.21^{b}	3.44±1.08^{b}	3.28±1.16^{b}	3.48±1.09^{bc}	7.66±2.02^{ab}
93.5mg/kgbwt	*Persea Americana*	13.30±1.57^{a}	8.60±1.17^{ab}	7.80±1.02^{a}	6.72±0.37^{a}	6.00±0.35^{a}	5.00±0.41^{ab}	9.00±1.19^{ab}

Results are expressed as Means ± SD for five mice per group. Values followed by the same superscript are not statistically different ($p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test.

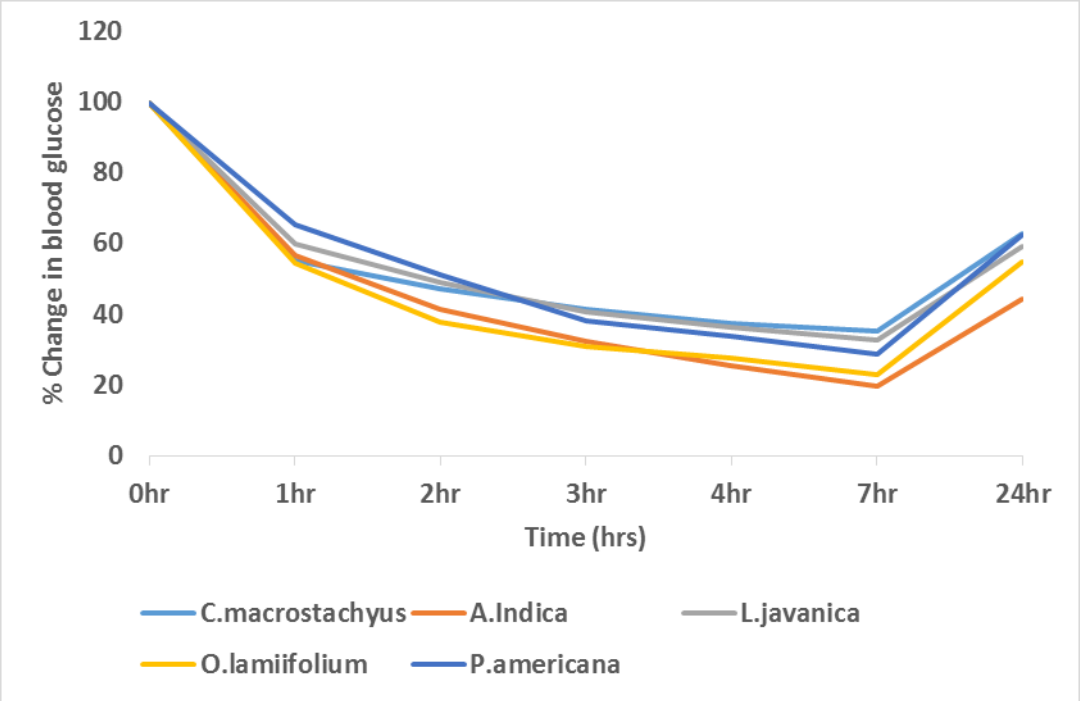

Figure 4.15: A comparison of mean percentage change in blood glucose levels of mice intraperitoneally administered with extracts at 93.5mg/kg body weight

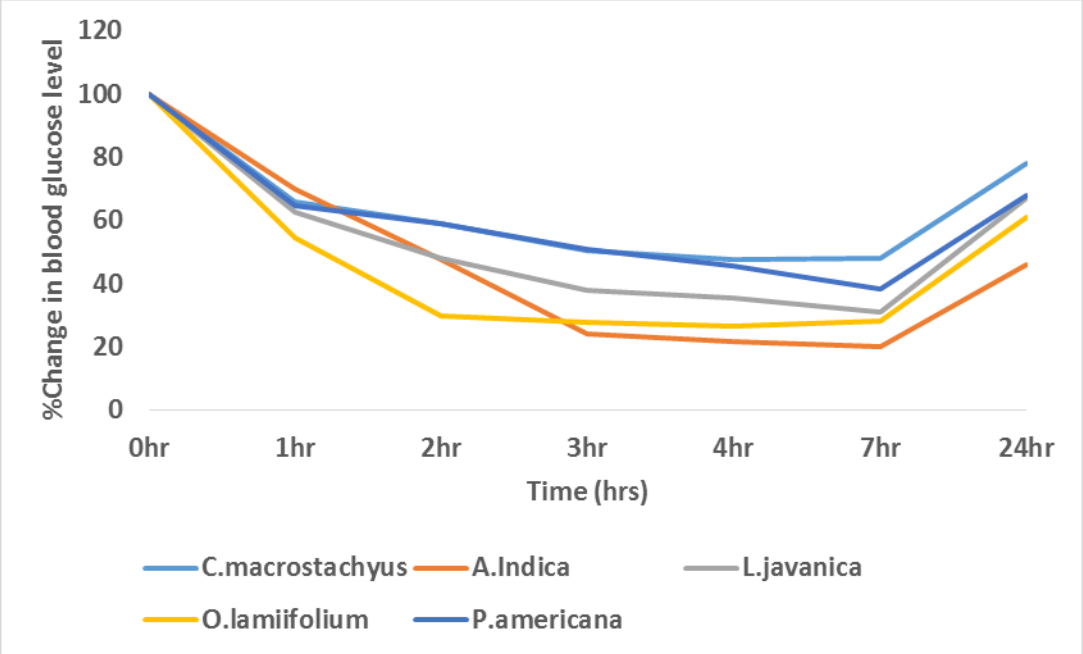

Figure 4.16: A comparison of mean percentage change in blood glucose levels of mice orally administered with extracts at 93.5mg/kg body weight

Tables 17 and 18 shows the hypoglycemic effect of intraperitoneal and oral administration of 180.9mg/kg body weight of different plant extracts in mice. Results indicates that the aqueous leaf extracts of five plants lowered the blood glucose levels in alloxan induced diabetic mice through both routes. The optimal dose for all the extracts in both routes occurred between the third and the seventh hour where the sugar reduction rate was between 51% and 84%. By the third hour, the intraperitoneal administration of *A. indica* had recorded the highest reduction rate in the blood glucose levels (68%) which remained persistently high upto the seventh hour (84%) (Figure 4.17).

In the third hour, oral administration of the extracts demonstrated the percent reductions in blood glucose levels at 45.90%, 28.04%, 41.32%, 37.17% and 47.49% for *C. macrostachyus, A. indica, L. javanica, O. lamiifolium and P. americana* respectively (Figure 4.18). By the seventh hour, blood sugar levels had been reduced to 52.0%, 20.93%, 32.70%, 29.30% and 37.28% for *C. macrostachyus, A. indica, L. javanica, O. lamiifolium and P. americana* respectively (Figure 4.18). The reductions in blood sugar levels persisted upto the seventh hour, in the twenty fourth hour, the mice had regained their diabetic states.

Table 4.17: Antidiabetic effect of intraperitoneal administration of the five aqueous plant extracts in mice at 180.9mg/kg body weight dose

Treatment	Plant Extracts	Time (hrs)						
		0hr	1hr	2hr	3hr	4hr	7hr	24hr
180.9mg/kgbwt	*Croton macrostachyus*	12.50±1.02^{a}	9.42±1.04^{a}	7.28±0.98^{a}	6.16±0.23^{ab}	5.48±0.34^{a}	5.02±0.15^{a}	7.84±0.59^{ab}
180.9mg/kgbwt	*Azardiratchta indica*	13.14±3.79^{a}	7.82±1.45^{a}	5.54±0.90^{a}	4.12±0.75^{b}	3.12±0.74^{b}	2.08±0.43^{b}	5.16±0.74^{b}
180.9mg/kgbwt	*Lippia javanica*	14.36±3.28^{a}	9.76±2.77^{a}	7.60±1.88^{a}	5.94±1.38^{ab}	5.24±0.72^{a}	4.60±0.68^{a}	9.08±1.18^{a}
180.9mg/kgbwt	*Ocimum lamiifolium*	15.90±5.49^{a}	10.60±4.66^{a}	7.92±2.63^{a}	6.38±1.89^{a}	5.70±1.31^{a}	5.12±0.93^{a}	9.88±2.33^{a}
180.9mg/kgbwt	*Persea americana*	14.98±2.55^{a}	10.84±2.31^{a}	7.58±1.79^{a}	5.96±0.97^{ab}	5.16±0.38^{a}	4.80±0.38^{a}	10.18±1.55^{a}

Results are expressed as Means ± SD for five mice per group. Values followed by the same superscript are not statistically different ($p \leq 0.05$); analysed by ANOVA followed by Tukey's post hoc test.

Table 4.18: Antidiabetic effect of oral administration of the five aqueous plant extracts in mice at 180.9mg/kg body weight dose

Treatment	Plant Extracts	Time (hrs)						
		0hr	1hr	2hr	3hr	4hr	7hr	24hr
180.9mg/kgbwt	*Croton marostachyus*	9.14±1.11^{b}	6.78±1.70^{b}	5.32±1.20^{b}	4.20±1.11^{b}	4.44±0.90^{ab}	4.68±0.84^{a}	7.76±1.23^{a}
180.9mg/kgbwt	*Azardiratchta indica*	15.20±1.61^{a}	10.16±1.36^{ab}	6.74±1.24^{ab}	4.24±0.64^{b}	3.84±0.74^{b}	3.18±0.80^{b}	7.38±1.60^{a}
180.9mg/kgbwt	*Lippia javanica*	14.00±2.50^{a}	9.88±2.41^{ab}	7.26±1.45^{ab}	5.78±1.00^{ab}	5.22±0.92^{ab}	4.54±0.60^{a}	8.90±1.53^{a}
180.9mg/kgbwt	*Ocimum lamiifolium*	17.38±3.52^{a}	11.32±1.88^{a}	6.94±0.96^{ab}	6.36±0.86^{a}	5.28±0.55^{a}	4.92±0.22^{a}	8.26±1.08^{a}
180.9mg/kgbwt	*Persea americana*	13.46±1.76^{a}	8.60±1.47^{ab}	7.66±1.12^{a}	6.34±0.48^{a}	5.56±0.51^{a}	4.96±0.11^{a}	8.70±1.62^{a}

Results are expressed as Means ± SD for five mice per group. Values followed by the same superscript are not statistically different ($p \leq 0.05$); analysed by ANOVA followed by Tukey's post hoc test

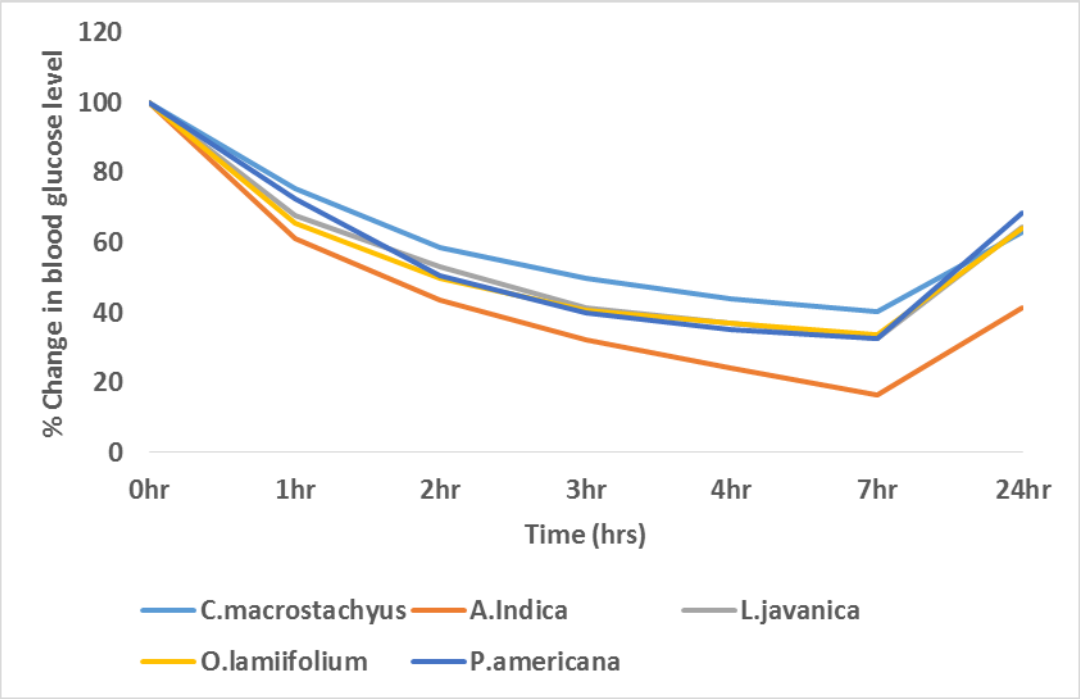

Figure 4. 17: A comparison of mean percentage change in blood glucose levels of mice intraperitoneally administered with extracts at 180.9mg/kg body weight

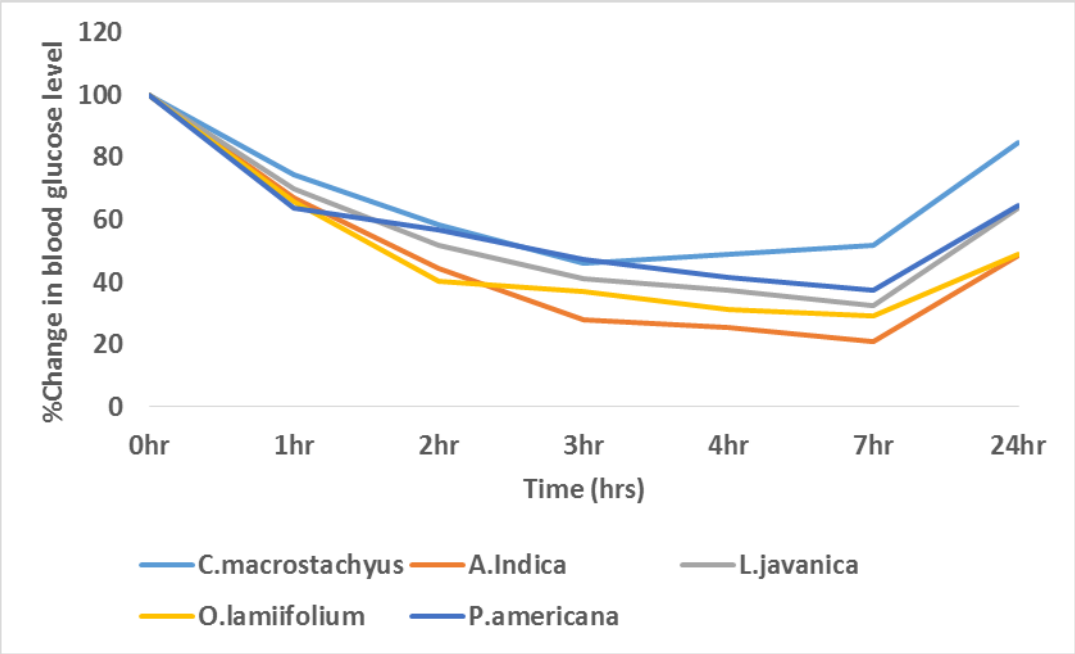

Figure 4. 18: A comparison of mean percentage change in blood glucose levels of mice orally administered with extracts at 180.9mg/kg body weight

Tables 19 and 20 shows the hypoglycemic effect of intraperitoneal and oral administration of 350mg/kg body weight of different medicinal plant extracts in mice models. Results show that in both routes, the optimal dose for all the extracts occurred between the third and the seventh hour where the sugar reduction rate was between 35% and 78%. By the third hour the percentage reductions in blood glucose levels were at 43.95%, 39.16%, 41.22%, 30.43% and 41.53% for *C. macrostachyus, A. indica, L. javanica, O. lamiifolium and P. americana* respectively (Figure 4.19). In the seventh hour *O. lamiifolium* had recorded the highest reductions in blood glucose levels by 78% followed *A. indica* by at 76% (Figure 4.19).

The oral administration of the extracts at the same dose in mice also lowered blood glucose levels appreciably from the first hour to the seventh hour with *A. indica* and *O. lamiifolium* recording lowest blood glucose levels by the seventh hour (the percentage reductions in glucose levels by this hour were at 22.64% and 25.79% respectively) (Figure 4.20). In the twenty fourth hour all the plant extracts had lost their hypoglycemic activity in both routes as mice had regained their respective diabetic states.

Table 4.19: Antidiabetic effect of intraperitoneal administration of the five aqueous plant extracts in mice at 350mg/kg body weight dose

Treatment	Plant Extracts	Time (hrs)						
		0hr	1hr	2hr	3hr	4hr	7hr	24hr
350mg/kgbwt	*Croton macrostachyus*	15.96±3.33^{ab}	11.36±2.76^{a}	8.88±2.09^{a}	6.94±1.30^{a}	5.68±0.98^{a}	5.14±0.70^{a}	6.64±0.55^{ab}
350mg/kgbwt	*Azardiratchta indica*	12.96±2.27^{b}	9.10±1.28^{a}	7.24±1.45^{a}	4.90±0.93^{b}	3.86±0.52^{b}	3.12±0.66^{b}	4.18±0.74^{c}
350mg/kgbwt	*Lippia javanica*	13.36±0.81^{ab}	8.36±0.61^{a}	6.46±0.51^{a}	5.50±0.44^{ab}	5.00±0.14^{ab}	4.24±0.23^{ab}	6.88±0.27^{ab}
350mg/kgbwt	*Ocimum lamiifolium*	17.94±3.42^{a}	9.58±1.30^{a}	6.62±0.61^{a}	5.38±0.71^{ab}	4.62±0.71^{ab}	4.04±0.84^{ab}	8.04±1.04^{a}
350mg/kgbwt	*Persea americana*	14.64±2.30^{ab}	10.14±1.94^{a}	7.68±1.33^{a}	6.02±0.97^{ab}	4.94±0.72^{ab}	4.04±0.53^{ab}	6.28±0.88^{b}

Results are expressed as Means ± SD for five mice per group. Values followed by the same superscript are not statistically different ($p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test.

Table 4.20: Antidiabetic effect of oral administration of the five aqueous plant extracts in mice at 350mg/kg body weight dose

Treatment	Plant Extracts	Time (hrs)						
		0hr	1hr	2hr	3hr	4hr	7hr	24hr
350mg/kgbwt	*Croton marostachyus*	12.72±3.47^{a}	9.66±2.74^{a}	8.74±1.95^{a}	8.18±2.03^{a}	7.34±1.34^{a}	6.62±0.69^{a}	9.90±1.27^{a}
350mg/kgbwt	*Azardiratchta indica*	14.88±2.84^{a}	10.12±0.97^{a}	7.48±1.45^{ab}	5.44±2.20^{ab}	4.32±1.25^{b}	3.12±0.71^{c}	7.40±1.73^{b}
350mg/kgbwt	*Lippia javanica*	14.18±1.02^{a}	9.08±1.04^{a}	7.16±0.65^{ab}	5.72±0.79^{ab}	5.02±0.30^{b}	4.20±0.24^{bc}	6.74±0.43^{b}
350mg/kgbwt	*Ocimum lamiifolium*	14.32±2.47^{a}	8.52±1.70^{a}	5.50±1.97^{b}	4.88±1.32^{b}	4.20±0.94^{b}	3.70±0.96^{bc}	5.12±0.66^{b}
350mg/kgbwt	*Persea americana*	12.80±0.99^{a}	9.58±1.56^{a}	7.56±0.98^{ab}	6.26±0.64^{ab}	4.92±0.50^{b}	4.50±0.67^{c}	6.94±1.71^{b}

Results are expressed as Means ± SD for five mice per group. Values followed by the same superscript are not statistically different ($p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test.

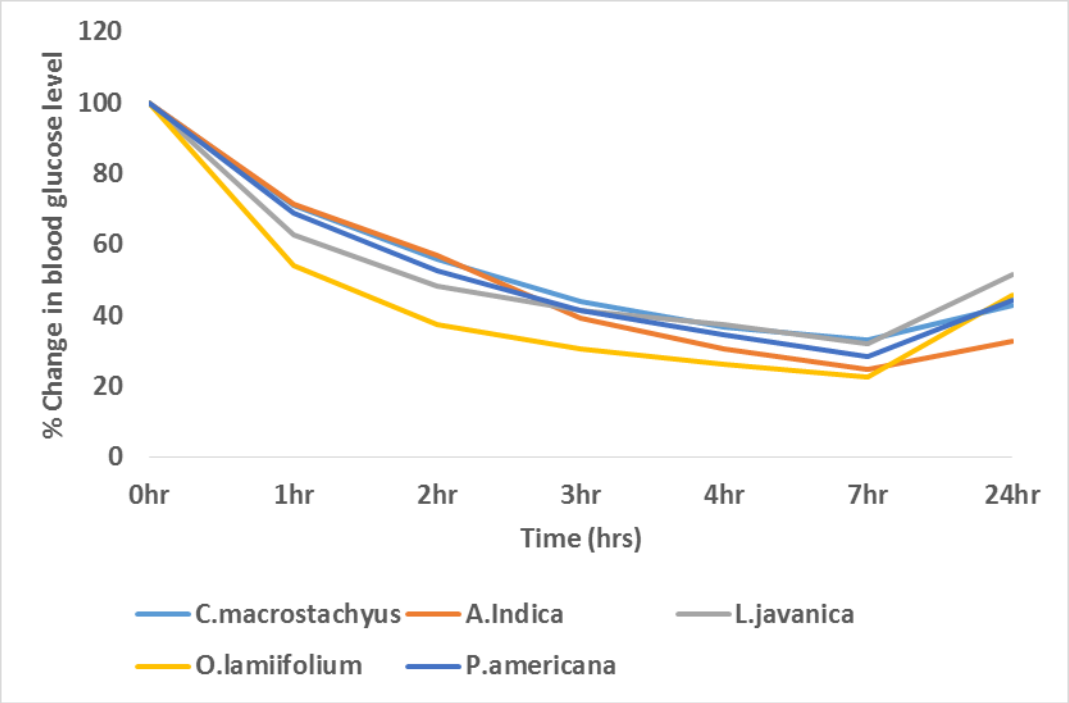

Figure 4.19: A comparison of mean percentage change in blood glucose levels of mice intraperitoneally administered with extracts at 350mg/k body weight

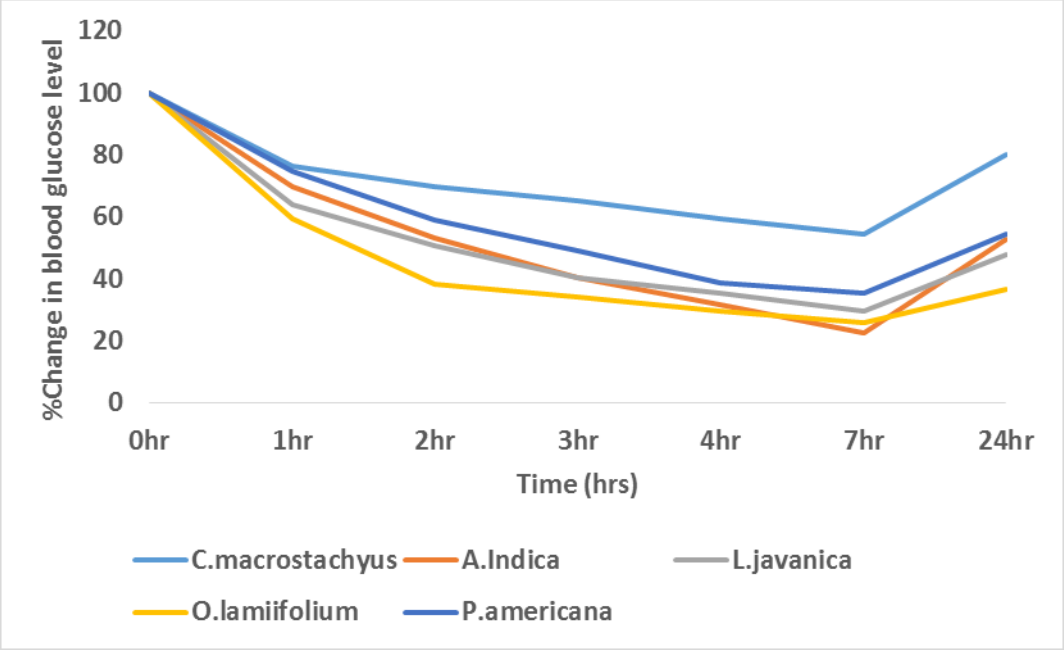

Figure 4.20: A comparison of mean percentage change in blood glucose levels of mice orally administered with extracts at 350mg/kg body weight

4.2 Evaluation of safety of orally and intraperitoneally administered aqueous plant extracts

4.2.1 Effect of intraperitoneally and orally administrered plant extracts on body weights of laboratory mice

Tables 4.21-22 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *C. macrostachyus* aqueous plant extracts for 28 days on body weights of laboratory mice. Results show that during the four week period, intraperitoneal and oral administration of *Croton macrostachyus* to mice for 28 days led to a significant ($p \leq 0.05$) lower rate of weekly weight gain relative to the control mice with 1000 mg/kg body weight recording the lowest values. In spite of this, the plant extract generally showed a steady weight gain throughout the four week period in both routes (Tables 4.21-22).

Table 4.21: Effect of intraperitoneal administration of aqueous leaf extract of *Croton macrostachyus* for 28 days on body weight of laboratory mice

Treatment (mg/kgbw)	Weekly weight of mice (g)					ΔWeight/Week (g/Week)
	0	1	2	3	4	
Control	20.80 ± 0.84^{b}	21.52 ± 0.79^{b}	22.04 ± 0.74^{b}	23.12 ± 0.53^{a}	23.64 ± 0.40^{a}	0.71 ± 0.12^{a}
450	22.00 ± 1.22^{ba}	22.82 ± 1.17^{ba}	23.34 ± 1.38^{ba}	23.84 ± 0.91^{a}	24.38 ± 1.56^{a}	0.60 ± 0.10^{ba}
670	22.40 ± 1.00^{ba}	22.94 ± 1.02^{ba}	23.56 ± 0.98^{ba}	24.14 ± 1.59^{a}	24.52 ± 0.91^{a}	0.53 ± 0.15^{ba}
1000	23.40 ± 1.14^{a}	23.86 ± 1.21^{a}	24.30 ± 1.28^{b}	24.82 ± 1.25^{a}	25.23 ± 1.22^{a}	0.46 ± 0.02^{b}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. **Key -Δ-represents change in**

Table 4.22: Effect of oral administration of aqueous leaf extract of *Croton macrostachyus* for 28 days on body weight of laboratory mice

Treatment (mg/kgbw)	Weekly weight of mice (g)					ΔWeight/Week (g/Week)
	0	1	2	3	4	
Control	20.60 ± 0.89^{b}	22.70 ± 0.62^{a}	23.74 ± 0.65^{a}	24.58 ± 0.77^{a}	25.32 ± 0.76^{a}	1.18 ± 0.20^{a}
450	21.80 ± 1.30^{ba}	22.54 ± 1.44^{a}	23.44 ± 1.08^{a}	24.26 ± 1.00^{a}	25.00 ± 1.15^{a}	0.80 ± 0.38^{ba}
670	23.00 ± 1.58^{a}	23.70 ± 1.52^{a}	24.32 ± 1.49^{a}	24.82 ± 1.44^{a}	25.36 ± 1.46^{a}	0.59 ± 0.04^{b}
1000	23.60 ± 1.14^{a}	24.08 ± 1.09^{a}	24.42 ± 1.11^{a}	24.82 ± 1.13^{a}	25.68 ± 1.53^{a}	0.52 ± 0.23^{b}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. **Key -Δ-represents change in**

Tables 4.23-24 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Azadiratchta indica* aqueous plant extract for 28 days on body weights of laboratory mice. Results show that during the four week period, the intraperitoneal and oral administration of *A. indica* to mice for 28 days showed a steady weight gain throughout the four week period in all the four treatments. However, a significant ($p \leq 0.05$) decrease in the rate of weekly weight gain as indicated was dose related with 1000 mg/kg body weight of the aqueous leaf extract recording the lowest rate of weekly weight gain.

Table 4.23: Effect of intraperitoneal administration of aqueous leaf extract of *Azadiratchta indica* for 28 days on body weight of laboratory mice

Treatment (mg/kgbw)	Weekly weight of mice (g)					ΔWeight/Week (g/Week)
	0	1	2	3	4	
Control	20.80 ± 0.84^{b}	21.52 ± 0.79^{b}	22.04 ± 0.74^{b}	23.12 ± 0.53^{a}	23.64 ± 0.40^{a}	0.71 ± 0.12^{a}
450	21.80 ± 1.30^{ba}	22.92 ± 1.51^{ba}	23.52 ± 1.48^{ba}	24.38 ± 1.13^{ba}	24.36 ± 1.30^{a}	0.64 ± 0.32^{a}
670	23.40 ± 1.52^{a}	24.36 ± 1.19^{a}	24.84 ± 1.08^{a}	25.10 ± 1.39^{a}	25.55 ± 1.42^{a}	0.53 ± 0.17^{b}
1000	23.80 ± 1.30^{a}	24.38 ± 1.25^{a}	24.74 ± 1.29^{a}	25.08 ± 1.48^{a}	25.06 ± 1.30^{a}	0.32 ± 0.28^{c}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. **Key -Δ-represents change in**

Table 4.24: Effect of oral administration of aqueous leaf extract of *Azadiratchta indica* for 28 days on body weight of laboratory mice

Treatment (mg/kgbw)	Weekly weight of mice (g)					ΔWeight/Week (g/Week)
	0	1	2	3	4	
Control	20.60 ± 0.89^{b}	22.70 ± 0.62^{a}	23.74 ± 0.65^{a}	24.58 ± 0.77^{a}	25.32 ± 0.76^{a}	1.18 ± 0.20^{a}
450	21.20 ± 1.30^{a}	21.78 ± 1.17^{a}	24.02 ± 1.25^{a}	24.59 ± 0.77^{a}	25.32 ± 0.76^{a}	1.03 ± 0.07^{a}
670	23.00 ± 1.22^{a}	23.56 ± 1.28^{a}	23.86 ± 1.70^{a}	24.58 ± 1.32^{a}	25.24 ± 1.30^{a}	0.56 ± 0.05^{b}
1000	22.80 ± 1.92^{a}	23.36 ± 1.81^{a}	23.74 ± 0.64^{a}	24.34 ± 1.63^{a}	24.84 ± 1.57^{a}	0.51 ± 0.09^{b}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. **Key -Δ-represents change in**

Tables 4.25-26 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Lippia javanica* aqueous plant extract for 28 days on body weights of laboratory mice.

Results show that during the four week period, intraperitoneal and oral administration of *L. javanica* to mice for 28 days led to a significant ($p \leq 0.05$) lower rate of weekly weight gain as the dosage increased across treatments with a dose level of 1000 mg/kg body weight recording the lowest rate of weekly weight gain. In spite of this, the plant extract generally showed a steady weight gain throughout the four week period in both routes as observed from values of the previous week (Tables 4.25-26). However, during the third and the fourth week the extract showed no significant difference in the rate of weekly weight gain of laboratory mice in both routes at $p \leq 0.05$.

Table 4.25: Effect of intraperitoneal administration of aqueous leaf extract of *Lippia javanica* for 28 days on body weight of laboratory mice

Treatment (mg/kgbw)	Weekly weight of mice (g)					ΔWeight/Week (g/Week)
	0	1	2	3	4	
Control	20.80 ± 0.84[b]	21.52 ± 0.79[b]	22.04 ± 0.74[b]	23.12 ± 0.53[a]	23.64 ± 0.40[a]	0.71±0.12[a]
450	22.40±1.14[a]	22.96±1.74[a]	22.52±1.77[a]	23.92±1.76[a]	24.62±0.84[a]	0.56±0.15[ab]
670	22.41±1.82[a]	23.00±1.92[a]	23.92±1.62[a]	24.06±1.62[a]	24.62±1.79[a]	0.55±0.10[ab]
1000	21.80±1.79[a]	22.42±1.10[a]	23.44±0.98[a]	23.40±0.93[a]	23.92±1.75[a]	0.53±0.11[ab]

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤0.05; analysed by ANOVA followed by Tukey's post hoc test. **Key -Δ-represents change in**

Table 4.26: Effect of oral administration of aqueous leaf extract of *Lippia javanica* for 28 days on body weight of laboratory mice

Treatment (mg/kgbw)	Weekly weight of mice (g)					ΔWeight/Week (g/Week)
	0	1	2	3	4	
Control	20.60 ± 0.89[b]	22.70 ± 0.62[a]	23.74 ± 0.65[a]	24.58 ± 0.77[a]	25.32 ± 0.76[a]	1.18±0.20[a]
450	22.20±1.92[a]	22.84±1.83[a]	23.74±1.62[a]	24.06±1.62[a]	25.62±1.79[a]	0.86±0.42[ba]
670	22.00±1.22[a]	22.62±1.19[a]	23.52±1.13[a]	24.58±0.77[a]	24.30±1.19[a]	0.58±0.06[b]
1000	21.40±1.67[a]	22.50±1.54[a]	23.14±0.65[a]	23.72±1.13[a]	23.64±1.45[a]	0.56±0.08[b]

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤0.05; analysed by ANOVA followed by Tukey's post hoc test. **Key -Δ-represents change in**

Tables 4.27-28 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Ocimum lamiifolium* aqueous plant extract for 28 days on body weights of laboratory mice. Results show that during the four week period, intraperitoneal and oral administration of *O. lamiifolium* to mice for 28 days showed a steady weight gain throughout the four week period in all the four treatments. However, a dose dependent significant ($p \leq 0.05$) decrease in the rate of weekly weight gain was indicated with 1000 mg/kg body weight dose of the aqueous leaf extract recording the lowest change in both routes. Oral administration of the extract showed no statistical difference ($p \leq 0.05$) in weight gain of mice during the third and fourth week.

Table 4.27: Effect of intraperitoneal administration of aqueous leaf extract of *Ocimum lamiifolium* for 28 days on body weight of laboratory mice

Treatment (mg/kgbw)	Weekly weight of mice (g) 0	1	2	3	4	ΔWeight/Week (g/Week)
Control	20.80 ± 0.84 ^{b}	21.52 ± 0.79^{b}	22.04 ± 0.74^{b}	23.12 ± 0.53 ^{a}	23.64 ± 0.40 ^{a}	0.71±0.12 ^{a}
450	22.00±1.58^{bc}	22.64±1.48^{bc}	23.28±1.36^{bc}	24.06±0.74^{bc}	24.54±1.17^{bc}	0.64±0.23 ^{a}
670	23.00±1.22^{ba}	23.94±0.89^{ba}	24.50±0.82^{ba}	24.94±0.76^{ba}	25.42±0.70^{ba}	0.61±0.18 ^{ab}
1000	24.30±0.84^{a}	24.88±0.85^{a}	25.38±0.85^{a}	26.12±0.27^{a}	26.64±0.75^{a}	0.59±0.16 ^{c}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤0.05; analysed by ANOVA followed by Tukey's post hoc test. **Key -Δ-represents change in**

Table 4.28: Effect of oral administration of aqueous leaf extract of *Ocimum lamiifolium* for 28 days on body weight of laboratory

Treatment (mg/kgbw)	Weekly weight of mice (g) 0	1	2	3	4	ΔWeight/Week (g/Week)
Control	20.60 ± 0.89^{b}	22.70 ± 0.62 ^{a}	23.74 ± 0.65 ^{a}	24.58 ± 0.77 ^{a}	25.32 ± 0.76 ^{a}	1.18±0.20 ^{a}
450	22.40±0.55^{ba}	23.00±0.56^{ba}	23.74±0.65^{ba}	24.58±0.77 ^{a}	25.32±0.76 ^{a}	0.73±0.26^{b}
670	22.00±1.58^{ba}	22.70±1.45^{ba}	23.58±0.54^{ba}	24.30±0.64 ^{a}	24.80±0.60 ^{a}	0.70±0.17^{b}
1000	23.80±1.30^{a}	24.42±1.26^{a}	24.96±1.34^{a}	25.50±1.35 ^{a}	26.00±1.29 ^{a}	0.55±0.20^{b}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤0.05; analysed by ANOVA followed by Tukey's post hoc test. **Key -Δ-represents change in**

Tables 4.29-30 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Persea americana* aqueous plant extract for 28 days on body weights of laboratory mice. Results show that during the four week period, intraperitoneal and oral administration of *P. americana* to mice for 28 days led to a significant ($p \leq 0.05$) lower rate of weekly weight gain as the dosage increased across treatments with a dose level of 1000 mg/kg body weight recording the lowest rate of weekly weight gain (Tables 4.29-30).

Table 4.29: Effect of intraperitoneal administration of aqueous leaf extract of *Persea americana* for 28 days on body weight of laboratory mice

Treatment (mg/kgbw)	Weekly weight of mice (g)					ΔWeight/Week (g/Week)
	0	1	2	3	4	
Control	20.80 ± 0.84 [b]	21.52 ± 0.79[b]	22.04 ± 0.74[b]	23.12 ± 0.53 [a]	23.64 ± 0.40 [a]	0.71±0.26 [a]
450	23.20±1.92 [a]	24.08±1.81 [a]	24.86±1.84 [a]	25.26±1.76 [a]	26.22±1.85 [a]	0.76±0.25 [a]
670	22.40±1.52 [a]	23.12±1.41 [a]	23.74±1.40[ba]	24.64±1.25[ba]	25.28±1.16[ba]	0.72±0.12 [a]
1000	22.00±1.58 [a]	22.70±1.51 [a]	23.28±1.40[ba]	24.08±1.44[ba]	24.76±1.53[ba]	0.69±0.09 [a]

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤0.05; analysed by ANOVA followed by Tukey's post hoc test. **Key -Δ-represents change in**

Table 4.30: Effect of oral administration of aqueous leaf extract of *Persea americana* for 28 days on body weight of laboratory mice

Treatment (mg/kgbw)	Weekly weight of mice (g)					ΔWeight/Week (g/Week)
	0	1	2	3	4	
Control	20.60 ± 0.89[b]	22.70 ± 0.62 [a]	23.74 ± 0.65 [a]	24.58 ± 0.77 [a]	25.32 ± 0.76 [a]	1.18±0.20 [a]
450	22.00±1.67[ba]	22.70±1.59 [a]	23.74±1.52 [a]	24.58±0.77 [a]	25.32±1.47 [a]	0.83±0.15[ba]
670	22.60±1.58[ba]	23.66±1.53 [a]	23.98±1.52 [a]	24.72±1.58 [a]	25.32±0.76 [a]	0.68±0.31[b]
1000	23.20±0.84 [a]	23.94±0.80 [a]	24.56±0.72 [a]	25.18±0.77 [a]	25.68±0.79 [a]	0.62±0.10[b]

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤0.05; analysed by ANOVA followed by Tukey's post hoc test. **Key -Δ-represents change in**

4.2.2 Effect of intraperitoneally and orally administrered plant extracts on organ weights of laboratory mice.

Tables 4.31-32 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Croton macrostachyus* aqueous leaf extract for 28 days on organ weights of laboratory mice. Results show that across treatments, the intraperitoneal and oral administration of *C. macrostachyus* to mice for 28 days did not significantly ($p \leq 0.05$) change the organ weights of brain, liver, kidney, spleen, lungs and heart. However, the intraperitoneal administration of aqueous leaf extracts of *C. macrostachyus* at a dose level of 1000 mg/kg body weight showed a significant ($p \leq 0.05$) increase in the weight of testes relative to the control mice.

Table 4.31: Effect of intraperitoneal administration of aqueous leaf extract of *Croton macrostachyus* for 28 days on organ weights of laboratory mice

Treatment (mg/kgbw)	Organ weights of laboratory mice (g)						
	Brain	**Liver**	**Kidney**	**Spleen**	**Lungs**	**Testes**	**Heart**
Control	0.53± 0.05 [a]	2.21± 0.30 [a]	0.44± 0.08 [a]	0.31± 0.06 [a]	0.40± 0.08 [a]	0.10± 0.02 [b]	0.11± 0.01 [a]
450	0.54± 0.05 [a]	2.24± 0.29 [a]	0.45± 0.07 [a]	0.33± 0.05 [a]	0.41± 0.10 [a]	0.10± 0.02 [b]	0.12± 0.02 [a]
670	0.55± 0.03 [a]	2.34± 0.28 [a]	0.46± 0.08 [a]	0.34± 0.06 [a]	0.43± 0.06 [a]	0.11± 0.01 [b]	0.12± 0.02 [a]
1000	0.56± 0.03 [a]	2.38± 0.25 [a]	0.46± 0.06 [a]	0.37± 0.04 [a]	0.45± 0.07 [a]	0.24± 0.02 [a]	0.13± 0.09 [a]

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test.

Table 4.32: Effect of oral administration of aqueous leaf extract of *Croton macrostachyus* for 28 days on organ weights of laboratory mice

Treatment (mg/kgbw)	Organ weights of laboratory mice (g)						
	Brain	**Liver**	**Kidney**	**Spleen**	**Lungs**	**Testes**	**Heart**
Control	0.52± 0.05[a]	1.53± 0.24 [a]	0.33± 0.05 [a]	0.19± 0.03 [a]	0.34± 0.04 [a]	0.10± 0.03 [a]	0.11± 0.01 [a]
450	0.53± 0.04 [a]	1.54± 0.21 [a]	0.35± 0.06 [a]	0.21± 0.04 [a]	0.35± 0.05 [a]	0.11± 0.07 [a]	0.11± 0.01 [a]
670	0.54± 0.06 [a]	1.63± 0.19 [a]	0.37± 0.05 [a]	0.22± 0.05 [a]	0.35± 0.04 [a]	0.11± 0.02 [a]	0.11± 0.01 [a]
1000	0.54± 0.05 [a]	1.72± 0.20 [a]	0.38± 0.04 [a]	0.22± 0.02 [a]	0.36± 0.04 [a]	0.12± 0.02 [a]	0.11± 0.02 [a]

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test.

Tables 4.33-34 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Azadiratchta indica* aqueous leaf extract for 28 days on organ weights of laboratory mice. Results show that the intraperitoneal and oral administration of *A. indica* to mice for 28 days did not significantly ($p \leq 0.05$) change the organ weights of brain, liver, kidney, spleen, lungs and heart across the four treatments. However, the intraperitoneal administration of the extract at 1000 mg/kg body weight showed a significant ($p \leq 0.05$) difference in the weight of testes relative to the control mice and those mice treated at 450 mg/kg and 670mg/kg body weight.

Table 4.33: Effect of intraperitoneal administration of aqueous leaf extract of *Azadiratchta indica* for 28 days on organ weights of laboratory mice

Treatment (mg/kgbw)	Organ weights of laboratory mice (g)						
	Brain	**Liver**	**Kidney**	**Spleen**	**Lungs**	**Testes**	**Heart**
Control	0.53± 0.05 [a]	2.21± 0.30 [a]	0.41± 0.09 [a]	0.31± 0.06 [a]	0.40± 0.08 [a]	0.10± 0.02 [b]	0.13± 0.09 [a]
450	0.53± 0.02 [a]	2.26± 0.33 [a]	0.45± 0.09 [a]	0.32± 0.05 [a]	0.41± 0.05 [a]	0.10± 0.02 [b]	0.14± 0.05 [a]
670	0.55± 0.01 [a]	2.40± 0.19 [a]	0.46± 0.08 [a]	0.33± 0.06 [a]	0.43± 0.07 [a]	0.11± 0.01 [b]	0.14± 0.02 [a]
1000	0.63± 0.27 [a]	2.45± 0.25 [a]	0.47± 0.07 [a]	0.34± 0.05 [a]	0.45± 0.07 [a]	0.24± 0.02 [a]	0.15± 0.02 [a]

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test.

Table 4.34: Effect of oral administration of aqueous leaf extract of *Azadiratchta indica* for 28 days on organ weights of laboratory mice

Treatment (mg/kgbw)	Organ weights of laboratory mice (g)						
	Brain	**Liver**	**Kidney**	**Spleen**	**Lungs**	**Testes**	**Heart**
Control	0.52± 0.05[a]	1.53± 0.24 [a]	0.33± 0.05 [a]	0.19± 0.03 [a]	0.33± 0.06 [a]	0.11± 0.03 [a]	0.11± 0.01 [a]
450	0.54± 0.04 [a]	1.54± 0.21 [a]	0.33± 0.06 [a]	0.20± 0.03 [a]	0.34± 0.04 [a]	0.11± 0.02 [a]	0.12± 0.01 [a]
670	0.54± 0.05 [a]	1.65± 0.19 [a]	0.36± 0.05 [a]	0.22± 0.04 [a]	0.36± 0.03 [a]	0.12± 0.01 [a]	0.12± 0.02 [a]
1000	0.52± 0.06 [a]	1.80± 0.31 [a]	0.38± 0.06 [a]	0.23± 0.06 [a]	0.37± 0.03 [a]	0.12± 0.02 [a]	0.13± 0.02 [a]

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test.

Tables 4.35-36 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Lippia javanica* aqueous leaf extract for 28 days on organ weights of laboratory mice. Results show that the intraperitoneal and oral administration of *L. javanica* to mice for 28 days did not significantly ($p \leq 0.05$) change the organ weights of brain, liver, kidney, spleen, lungs and heart across the four treatments. However, the intraperitoneal and oral administration of aqueous leaf extract of *Lippia javanica* at a dose level of 1000mg/kg body weight 28 days showed a significant increase in the weight of testes and spleen (orally) relative to those of the control mice.

Table 4.35: Effect of intraperitoneal administration of aqueous leaf extract of *Lippia javanica* for 28 days on organ weights of laboratory mice

Treatment (mg/kgbw)	Organ weights of laboratory mice (g)						
	Brain	**Liver**	**Kidney**	**Spleen**	**Lungs**	**Testes**	**Heart**
Control	0.51±0.03 [a]	2.21± 0.30 [a]	0.40±0.11 [a]	0.23±0.05 [b]	0.34±0.10 [a]	0.07±0.02 [c]	0.12±0.01 [a]
450	0.53± 0.05 [a]	2.27±0.28 [a]	0.44±0.07 [a]	0.31± 0.06 [a]	0.40± 0.08 [a]	0.08±0.01 [cb]	0.12±0.04 [a]
670	0.54±0.04 [a]	2.33±0.22 [a]	0.45±0.08 [a]	0.32±0.04 [a]	0.45±0.05 [a]	0.11±0.01 [a]	0.13± 0.09 [a]
1000	0.54±0.03 [a]	1.12±0.20 [b]	0.46± 0.08 [a]	0.33±0.02 [a]	0.46±0.08 [a]	0.24± 0.02 [a]	0.14±0.02 [a]

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test.

Table 4.36: Effect of oral administration of aqueous leaf extract of *Lippia javanica* for 28 days on organ weights of laboratory mice

Treatment (mg/kgbw)	Organ weights of laboratory mice (g)						
	Brain	**Liver**	**Kidney**	**Spleen**	**Lungs**	**Testes**	**Heart**
Control	0.52± 0.05[a]	1.40±0.41 [a]	0.33± 0.05 [a]	0.19± 0.03 [a]	0.33±0.04 [a]	0.05±0.04 [b]	0.11± 0.01 [a]
450	0.52±0.03 [a]	1.53± 0.24 [a]	0.32±0.04 [a]	0.20±0.04 [a]	0.34±0.06 [a]	0.10±0.02 [a]	0.11±0.02 [a]
670	0.52±0.07 [a]	1.54±0.20 [a]	0.32±0.04 [a]	0.22±0.02 [a]	0.34± 0.04 [a]	0.11±0.01 [a]	0.12±0.01 [a]
1000	0.53±0.02 [a]	1.65±0.20 [a]	0.34±0.04 [a]	0.24±0.05 [a]	0.35±0.02 [a]	0.12± 0.02 [a]	0.12±0.02 [a]

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test.

Tables 4.37-38 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Ocimum lamiifolium* aqueous leaf extract for 28 days on organ weights of laboratory mice. Results show that the intraperitoneal and oral administration of *O. lamiifolium* to mice for 28 days did not significantly ($p \leq 0.05$) change the organ weights of brain, liver, kidney, spleen, lungs and heart across treatments. However, the intraperitoneal administration of the extract showed a significant ($p \leq 0.05$) increase in weight of testes relative to the control mice at 1000mg/kg body weight.

Table 4.37: Effect of intraperitoneal administration of aqueous leaf extract of *Ocimum lamiifolium* for 28 days on organ weights of laboratory mice

Treatment (mg/kgbw)	Organ weights of laboratory mice (g)						
	Brain	**Liver**	**Kidney**	**Spleen**	**Lungs**	**Testes**	**Heart**
Control	0.53±0.05 [a]	2.21± 0.30 [a]	0.44±0.06 [a]	0.31± 0.06 [a]	0.38±0.08 [a]	0.10±0.02 [b]	0.12±0.01 [a]
450	0.53± 0.05 [a]	2.23±0.29 [a]	0.45±0.09 [a]	0.31±0.06 [a]	0.40± 0.08 [a]	0.11±0.01 [b]	0.13± 0.09 [a]
670	0.54±0.02 [a]	2.29±0.32 [a]	0.46± 0.07 [a]	0.32±0.05 [a]	0.41±0.05 [a]	0.23±0.03 [a]	0.14±0.05 [a]
1000	0.56±0.02 [a]	2.46±0.17 [a]	0.46±0.06[a]	0.33±0.06 [a]	0.43±0.08 [a]	0.24± 0.02 [a]	0.15±0.02 [a]

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test.

Table 4.38: Effect of oral administration of aqueous leaf extract of *Ocimum lamiifolium* for 28 days on organ weights of laboratory mice

Treatment (mg/kgbw)	Organ weights of laboratory mice (g)						
	Brain	**Liver**	**Kidney**	**Spleen**	**Lungs**	**Testes**	**Heart**
Control	0.52± 0.05[a]	1.53± 0.24 [a]	0.33± 0.05 [a]	0.19± 0.03 [a]	0.32±0.09 [a]	0.11±0.03 [a]	0.11± 0.01 [a]
450	0.52±0.05 [a]	1.53±0.24 [a]	0.33±0.06 [a]	0.20±0.03 [a]	0.34±0.06 [a]	0.11±0.01 [a]	0.11±0.02 [a]
670	0.53±0.05 [a]	1.54±0.21 [a]	0.34±0.04 [a]	0.20±0.03 [a]	0.34± 0.04 [a]	0.12± 0.02 [a]	0.12±0.02 [a]
1000	0.54±0.05 [a]	1.66±0.19 [a]	0.36±0.05 [a]	0.23±0.05 [a]	0.36±0.03 [a]	0.12±0.02 [a]	0.12±0.01 [a]

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test.

Tables 4.39-40 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Persea americana* aqueous plant extract for 28 days on organ weights of laboratory mice. Results show that across treatments, the intraperitoneal and oral administration of *P. americana* to mice for 28 days did not significantly ($p \leq 0.05$) change the organ weights of brain, liver, kidney, spleen, lungs and heart However, the administration of the extract at 1000mg/kg body weight showed a significant ($p \leq 0.05$) increase in the weight of testes (intraperitoneally) and spleen (orally) relative to those of the control mice.

Table 4.39: Effect of intraperitoneal administration of aqueous leaf extract of *Persea americana* for 28 days on organ weights of laboratory mice

Treatment (mg/kgbw)	Organ weights of laboratory mice (g)						
	Brain	**Liver**	**Kidney**	**Spleen**	**Lungs**	**Testes**	**Heart**
Control	0.53± 0.05 [a]	2.21± 0.30 [a]	0.46± 0.08 [a]	0.31± 0.06 [a]	0.40± 0.08 [a]	0.24± 0.02 [a]	0.13± 0.09 [a]
450	0.53±0.02 [a]	2.26±0.26 [a]	0.45±0.09 [a]	0.33±0.04 [a]	0.45±0.04 [a]	0.11±0.03 [b]	0.18±0.15 [a]
670	0.54±0.03 [a]	2.29±0.12 [a]	0.39±0.04 [a]	0.35±0.02 [a]	0.46±0.03 [a]	0.10±0.05 [b]	0.12±0.01 [a]
1000	0.55±0.03 [a]	2.35±0.23 [a]	0.45±0.06 [a]	0.37±0.08 [a]	0.45±0.07 [a]	0.09±0.01 [b]	0.13±0.01 [a]

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test.

Table 4.40: Effect of oral administration of aqueous leaf extract of *Persea americana* for 28 days on organ weights of laboratory mice

	Organ weights of laboratory mice (g)						
	Brain	**Liver**	**Kidney**	**Spleen**	**Lungs**	**Testes**	**Heart**
Control	0.52± 0.05[a]	1.53± 0.24 [a]	0.33± 0.05 [a]	0.19± 0.03 [b]	0.34± 0.04 [a]	0.12± 0.02 [a]	0.11± 0.01 [a]
450	0.53±0.04 [a]	1.54±0.19 [a]	0.34±0.04 [a]	0.22±0.03[ba]	0.34±0.06 [a]	0.12±0.01 [a]	0.11±0.01 [a]
670	0.54±0.03 [a]	1.66±0.20 [a]	0.36±0.05 [a]	0.23±0.02[ba]	0.36±0.02 [a]	0.11±0.03 [a]	0.12±0.02 [a]
1000	0.54±0.04 [a]	1.83±0.34 [a]	0.38±0.05 [a]	0.29±0.08 [a]	0.38±0.08 [a]	0.10±0.01 [a]	0.13±0.03 [a]

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test.

4.2.3 Effect of intraperitoneally and orally administrered plant extracts on the relative organ weight of laboratory mice

Tables 4.41-42 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Croton macrostachyus* aqueous leaf extract for 28 days on relative organ to body weight of laboratory mice. Results indicates that the intraperitoneal and oral administration of *C. macrostachyus* to mice for 28 days did not significantly ($p \leq 0.05$) alter the percent organ to body weight of the brain, liver, kidney, spleen, lungs and the heart across the four treatments. However, the intraperitoneal administration of 1000mg/kg body weight of the extract showed a significant ($p \leq 0.05$) increase in the percent organ to body weight of testes relative to the control mice.

Table 4.41: Effect of intraperitoneal administration of aqueous leaf extract of *Croton macrostachyus* for 28 days on relative organ weights of laboratory mice

Treatment (mg/kgbw)	Percent relative organ to body weight						
	Brain	**Liver**	**Kidney**	**Spleen**	**Lungs**	**Testes**	**Heart**
Control	2.06±0.22 [a]	8.65±1.30 [a]	1.78±0.35 [a]	1.21±0.24 [a]	1.56±0.31 [a]	0.42±0.08[b]	0.44±0.03 [a]
450	2.25±0.30 [a]	9.32±1.58 [a]	1.82±0.31[a]	1.35±0.19 [a]	1.69±0.42 [a]	0.42±0.05[b]	0.49±0.04 [a]
670	2.27±0.17 [a]	9.55±0.98 [a]	1.83±0.25[a]	1.39±0.30 [a]	1.76±0.30 [a]	0.46±0.03[b]	0.49±0.08 [a]
1000	2.41±0.10 [a]	10.29±1.23 [a]	1.97±0.25[a]	1.58±0.15 [a]	1.95±0.28 [a]	0.93±0.12 [a]	0.50±0.06 [a]

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test.

Table 4.42: Effect of oral administration of aqueous leaf extract of *Croton macrostachyus* for 28 days on relative organ weights of laboratory mice

Treatment (mg/kgbw)	Percent relative organ to body weight						
	Brain	**Liver**	**Kidney**	**Spleen**	**Lungs**	**Testes**	**Heart**
Control	2.03±0.13[a]	6.13±1.09 [a]	1.31±0.18 [a]	0.76±0.11 [a]	1.36±0.20 [a]	0.42±0.14 [a]	0.43±0.04 [a]
450	2.12±0.17 [a]	6.17±0.82 [a]	1.39±0.24 [a]	0.86±0.10 [a]	1.39±0.19 [a]	0.44±0.03 [a]	0.43±0.05 [a]
670	2.17±0.27 [a]	6.61±0.85 [a]	1.49±0.19 [a]	0.90±0.21 [a]	1.43±0.16 [a]	0.43±0.09 [a]	0.45±0.06 [a]
1000	2.31±0.42 [a]	7.31±0.63 [a]	1.64±0.34 [a]	0.89±0.23 [a]	1.52±0.16 [a]	0.49±0.07 [a]	0.47±0.09 [a]

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test.

Tables 4.43-44 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Azadiratchta indica* aqueous leaf extract for 28 days on relative organ to body weight of laboratory mice. Results show that across treatments, the intraperitoneal and oral administration of *Azadiratchta indica* to mice for 28 days did not significantly ($p \leq 0.05$) alter the percent organ to body weight of brain, liver, kidney, spleen, lungs, and the heart. However, the intraperitoneal and oral administration of 1000mg/kg body weight of the extract caused a significant ($p \leq 0.05$) increase in the percent organ to body weight of testes relative to those of the control mice.

Table 4.43: Effect of intraperitoneal administration of aqueous leaf extract of *Azadiratchta indica* for 28 days on relative organ weights of laboratory mice

Treatment (mg/kgbw)	Percent relative organ to body weight						
	Brain	**Liver**	**Kidney**	**Spleen**	**Lungs**	**Testes**	**Heart**
Control	2.06±0.22 [a]	8.65±1.30 [a]	1.72 ± 0.41 [a]	1.21±0.24 [a]	1.56±0.31 [a]	0.93±0.12 [b]	0.49±0.04 [a]
450	2.18 ± 0.17 [a]	9.26 ± 1.67 [a]	1.78±0.35 [a]	1.31 ± 0.21 [a]	1.67 ± 0.16 [a]	1.27 ± 0.24 [ba]	0.57 ± 0.25 [a]
670	2.27 ± 0.15 [a]	9.90 ± 0.76 [a]	1.84 ± 0.44 [a]	1.38 ± 0.29 [a]	1.76 ± 0.29 [a]	1.32 ± 0.21 [ba]	0.59 ± 0.12 [a]
1000	2.65 ± 0.94 [a]	10.42 ± 1.36 [a]	1.99 ± 0.36 [a]	1.46 ± 0.21 [a]	1.92 ± 0.26 [a]	1.36 ± 0.31 [a]	0.64 ± 0.12 [a]

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test.

Table 4.44: Effect of oral administration of aqueous leaf extract of *Azadiratchta indica* for 28 days on relative organ weights of laboratory mice

Treatment (mg/kgbw)	Percent relative organ to body weight						
	Brain	**Liver**	**Kidney**	**Spleen**	**Lungs**	**Testes**	**Heart**
Control	2.03±0.13 [a]	6.13±1.09 [a]	1.31±0.18 [a]	0.76±0.11 [a]	1.36±0.20 [a]	0.49±0.07 [b]	0.45±0.06 [a]
450	2.14±0.14 [a]	6.19 ± 1.04 [a]	1.34 ± 0.26 [a]	0.80 ± 0.16 [a]	1.36 ± 0.28 [a]	0.80 ± 0.13 [a]	0.47 ± 0.03 [a]
670	2.17±0.29 [a]	6.60 ± 0.46 [a]	1.43 ± 0.20 [a]	0.87 ± 0.14 [a]	1.44 ± 0.15 [a]	0.81 ± 0.10 [a]	0.49 ± 0.09 [a]
1000	2.17±0.20 [a]	7.51 ± 1.33 [a]	1.58 ± 0.32 [a]	0.97 ± 0.20 [a]	1.54 ± 0.19 [a]	0.91 ± 0.21 [a]	0.53 ± 0.12 [a]

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test.

Tables 4.45-46 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Lippia javanica* aqueous leaf extract for 28 days on relative organ to body weight of laboratory mice. Results show that intraperitoneal and oral administration of *L. javanica* to mice for 28 days did not significantly ($p \leq 0.05$) change the percent organ to body weight of brain, kidney, lungs, and the heart. In both routes, the extract caused a dose dependent significant ($p \leq 0.05$) increase in the percent organ to body weight that picked at 450mg/kg body weight of the liver, spleen and testes relative to that of control mice.

Table 4.45: Effect of intraperitoneal administration of aqueous leaf extract of *Lippia javanica* for 28 days on relative organ weights of laboratory mice

Treatment (mg/kgbw)	Percent relative organ to body weight						
	Brain	**Liver**	**Kidney**	**Spleen**	**Lungs**	**Testes**	**Heart**
Control	2.06±0.22 [a]	4.84±0.70 [b]	1.71±0.52 [a]	0.99±0.27 [b]	1.49±0.44 [a]	0.93±0.12 [b]	0.49±0.04 [a]
450	2.22±0.10 [a]	8.65±1.30 [a]	1.78±0.35 [a]	1.21±0.24 [ba]	1.56±0.31 [a]	1.33±0.11 [a]	0.50±0.18 [a]
670	2.28±0.27 [a]	9.39±1.30 [a]	1.84±0.34 [a]	1.34±0.22 [ba]	1.87±0.22 [a]	1.43±0.07 [a]	0.51±0.07 [a]
1000	2.26±0.26 [a]	9.81±0.67 [a]	1.94±0.34 [a]	1.38±0.08 [a]	1.94±0.31 [a]	1.57±0.33 [a]	0.59±0.10 [a]

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test.

Table 4.46: Effect of oral administration of aqueous leaf extract of *Lippia javanica* for 28 days on relative organ weights of laboratory mice

Treatment (mg/kgbw)	Percent relative organ to body weight						
	Brain	**Liver**	**Kidney**	**Spleen**	**Lungs**	**Testes**	**Heart**
Control	2.03±0.13 [a]	5.95±1.60 [a]	1.31±0.18 [a]	0.76±0.11 [b]	1.36±0.20 [a]	0.49±0.07 [b]	0.45±0.06 [a]
450	2.12±0.12 [a]	6.13±1.09 [a]	1.31±0.18 [a]	0.82±0.15 [ba]	1.38±0.25 [a]	0.88±0.11 [a]	0.45±0.09 [a]
670	2.19±0.09 [a]	6.26±0.99 [a]	1.35±0.22 [a]	0.92±0.06 [ba]	1.39±0.08 [a]	0.95±0.09 [a]	0.49±0.03 [a]
1000	2.21±0.35 [a]	6.85±0.94 [a]	1.43±019 [a]	1.02±0.20 [a]	1.47±0.10 [a]	1.19±0.35 [a]	0.49±0.10 [a]

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test.

Tables 4.47-48 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Ocimum lamiifolium* aqueous leaf extract for 28 days on relative organ to body weight of laboratory mice. Results show that intraperitoneal and oral administration of *O. lamiifolium* to mice for 28 days did not significantly ($p \leq 0.05$) alter the percent organ to body weight of the brain, liver, kidney, spleen, lungs and the heart across the four treatments. However, a dose dependent increase in the percent organ to body weight that picked at 670 mg/kg body weight of testes was indicated following the intraperitoneal administration of the extract.

Table 4.47: Effect of intraperitoneal administration of aqueous leaf extract of *Ocimum lamiifolium* for 28 days on relative organ weights of laboratory mice

Treatment (mg/kgbw)	Percent relative organ to body weight						
	Brain	**Liver**	**Kidney**	**Spleen**	**Lungs**	**Testes**	**Heart**
Control	2.06±0.22 [a]	8.65±1.30 [a]	1.78±0.35 [a]	1.21±0.24 [a]	1.56±0.31 [a]	0.41±0.06 [b]	0.49±0.04 [a]
450	2.20±0.15 [a]	9.35±1.50 [a]	1.80±0.25 [a]	1.32±0.21 [a]	1.56±0.29 [a]	0.44±0.04 [b]	0.51±0.06 [a]
670	2.18±0.22 [a]	9.21±1.26 [a]	1.85±0.35 [a]	1.27±0.24 [a]	1.69±0.25 [a]	0.93±0.12 [a]	0.59±0.21 [a]
1000	2.28±0.12 [a]	10.15±1.40 [a]	1.91±0.29 [a]	1.36±0.31 [a]	1.76±0.28 [a]	0.97±0.11 [a]	0.62±0.10 [a]

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test.

Table 4.48: Effect of oral administration of aqueous leaf extract of *Ocimum lamiifolium* for 28 days on relative organ weights of laboratory mice

Treatment (mg/kgbw)	Percent relative organ to body weight						
	Brain	**Liver**	**Kidney**	**Spleen**	**Lungs**	**Testes**	**Heart**
Control	2.03±0.13[a]	6.13±1.09 [a]	1.31±0.18 [a]	0.76±0.11 [a]	1.31±0.34 [a]	0.44±0.10 [a]	0.45±0.06 [a]
450	2.10±0.11 [a]	6.24±0.99 [a]	1.34±0.24 [a]	0.80±0.13 [a]	1.36±0.24 [a]	0.46±0.02 [a]	0.46±0.08 [a]
670	2.15±0.16 [a]	6.40±0.87 [a]	1.38±0.19 [a]	0.81±0.10 [a]	1.36±0.20 [a]	0.49±0.07 [a]	0.47±0.06 [a]
1000	2.19±0.22 [a]	6.72±1.06 [a]	1.46±0.18 [a]	0.91±0.21 [a]	1.45±0.10 [a]	0.49±0.06 [a]	0.48±0.04 [a]

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test.

Tables 4.49-50 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Persea americana* aqueous plant extract for 28 days on relative organ to body weights of laboratory mice. Results show that intraperitoneal and oral administration of *P. americana* to mice for 28 days did not significantly ($p \leq 0.05$) alter the percent organ to body weight of the brain, liver, kidney, spleen, lungs and the heart across the four. However, at 1000mg/kg body weight of extract indicated a significant ($p \leq 0.05$) increase in the percent organ to body weight of testes (intraperitoneally and orally) and spleen (orally) relative to the control mice.

Table 4.49: Effect of intraperitoneal administration of aqueous leaf extract of *Persea americana* for 28 days on relative organ weights of laboratory mice

Treatment (mg/kgbw)	Percent relative organ to body weight						
	Brain	**Liver**	**Kidney**	**Spleen**	**Lungs**	**Testes**	**Heart**
Control	2.06±0.22 [a]	8.65±1.30 [a]	1.78±0.35 [a]	1.21±0.24 [a]	1.56±0.31 [a]	0.37±0.03 [b]	0.49±0.04 [a]
450	2.19±0.21 [a]	9.31±1.65 [a]	1.62±0.24 [a]	1.33±0.11 [a]	1.84±0.19 [a]	0.43±0.24 [b]	0.50±0.04 [a]
670	2.24±0.24 [a]	9.44±0.30 [a]	1.84±0.42 [a]	1.43±0.07 [a]	1.90±0.17 [a]	0.44±0.11 [b]	0.56±0.09 [a]
1000	2.36±0.24 [a]	9.94±0.47 [a]	1.93±0.33 [a]	1.57±0.33 [a]	1.95±0.44 [a]	0.93±0.12 [a]	0.75±0.67 [a]

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test.

Table 4.50: Effect of oral administration of aqueous leaf extract of *Persea americana* for 28 days on relative organ weights of laboratory mice

Treatment (mg/kgbw)	Percent relative organ to body weight						
	Brain	**Liver**	**Kidney**	**Spleen**	**Lungs**	**Testes**	**Heart**
Control	2.03±0.13[a]	6.13±1.09 [a]	1.31±0.18 [a]	0.76±0.11 [b]	1.36±0.20 [a]	0.42±0.04 [a]	0.45±0.06 [a]
450	2.16±0.23 [a]	6.25±0.85 [a]	1.38±0.22 [a]	0.88±0.11[ba]	1.37±0.20 [a]	0.45±0.09 [a]	0.45±0.02 [a]
670	2.20±0.14 [a]	6.80±0.91 [a]	1.48±0.22 [a]	0.95±0.09 [ba]	1.46±0.09 [a]	0.47±0.03 [a]	0.48±0.11 [a]
1000	2.27±0.28 [a]	7.62±1.71 [a]	1.56±0.14 [a]	1.19±0.35 [a]	1.55±0.29 [a]	0.49±0.07 [b]	0.54±0.12 [a]

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test.

4.2.4 Determination of hematological profiles

4.2.4.1 Determination of Erythrocytes and related profiles

Tables 4.51-52 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Croton macrostachyus* aqueous leaf extract for 28 days on erythrocytes and related parameters in mice.

Results show that intraperitoneal and oral administration of *C. macrostachyus* to mice for 28 days did not significantly ($p \leq 0.05$) change the MCH levels across treatments. However, a dose dependent increase in RBC, Hb, MCV and PCV levels that picked at 670 mg/kg body weight was indicated across the four treatments. The oral and intraperitoneal administration of 1000mg/kg of aqueous leaf extracts recorded highest values of RBC, Hb, MCV and PCV and lowest values of MCH and MCHC relative to that of the control mice.

Table 4.51: Effect of intraperitoneal administration of aqueous leaf extract of *Croton macrostachyus* for 28 days on erythrocytes and related parameters in mice

Treatment (mg/kgbw)	Hematological parameters and indices					
	RBC (x10^6/µL)	Hb (g/dL)	PCV (%)	MCH (pg)	MCHC (%)	MCV (fL)
Control	9.28±0.91^{c}	7.96±0.78^{c}	43.20±3.29^{b}	8.65±1.26^{a}	35.22±2.04^{a}	27.22±2.38^{c}
450	9.54±1.22^{c}	10.30±1.06^{cb}	44.68±2.32^{ab}	10.95±1.94^{a}	32.02±1.33^{a}	36.33±1.19^{b}
670	12.76±1.76^{b}	12.90±2.47^{b}	48.08±3.14^{ab}	10.26±2.44^{a}	31.50±1.12^{a}	41.18±2.63^{a}
1000	18.00±2.07^{a}	16.80±1.63^{a}	50.18±3.49^{a}	9.10±2.73^{a}	21.52±3.1^{b}	42.80±2.06^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤0.05; analysed by ANOVA followed by Tukey's post hoc test. RBC = red blood cell count; Hb = hemoglobin; PCV = packed red cell volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume.

Table 4.52: Effect of oral administration of aqueous leaf extract of *Croton macrostachyus* for 28 days on erythrocytes and related parameters in mice

Treatment (mg/kgbw)	Hematological parameters and indices					
	RBC (x10^6/µL)	Hb (g/dL)	PCV (%)	MCH (pg)	MCHC (%)	MCV (fL)
Control	6.40±1.32^{c}	8.90±0.60^{c}	40.28±3.15^{c}	14.47±3.54^{a}	34.24±2.06^{a}	19.82±1.94^{c}
450	7.48±0.58^{c}	9.82±0.33^{cb}	41.92±1.46^{bc}	13.18±0.99^{a}	30.50±1.41^{ab}	32.8±21.35^{b}
670	11.90±1.21^{ba}	12.80±2.36^{b}	45.82±2.25^{ab}	10.90±2.48^{a}	27.84±3.45^{b}	41.30±2.65^{a}
1000	15.56±2.86^{a}	17.62±1.17^{a}	48.94±3.15^{a}	11.63±2.26^{a}	19.14±2.63^{c}	40.46±3.53^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤0.05; analysed by ANOVA followed by Tukey's post hoc test. RBC = red blood cell count; Hb = hemoglobin; PCV = packed red cell volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume.

Tables 4.53-54 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Azadiratchta indica* aqueous leaf extract for 28 days on erythrocytes and related parameters in mice. Results show that intraperitoneal administration of *A. indica* to mice for 28 days did not significantly ($p \leq 0.05$) change the MCH and MCHC levels across the four treatments. Orally MCH and MCHC were shown to decrease in a dose related manner. However, a dose dependent increase in RBC, Hb, MCV and PCV levels relative to the control mice was indicated in both routes.

Table 4.53: Effect of intraperitoneal administration of aqueous leaf extract of *Azadiratchta indica* for 28 days on erythrocytes and related parameters in mice

Treatment (mg/kgbw)	Hematological parameters and indices					
	RBC (x10^6/µL)	Hb (g/dL)	PCV (%)	MCH (pg)	MCHC (%)	MCV (fL)
Control	9.28±0.91^{c}	7.96±0.78^{c}	43.20±3.29^{b}	8.65±1.26^{a}	35.22±2.04^{a}	27.22±2.38^{c}
450	12.74±2.45^{b}	11.66±1.11^{cb}	44.82±2.37^{ab}	10.73±1.43^{a}	32.01±1.34^{a}	39.68±2.02^{b}
670	12.92±1.84^{b}	12.66±2.48^{b}	48.36±3.32^{ab}	10.00±0.72^{a}	31.49±1.11^{a}	40.80±3.16^{ab}
1000	19.10±0.67^{a}	16.40±2.01^{a}	50.26±3.48^{a}	10.20±1.30^{a}	21.53±3.14^{b}	44.50±1.00^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤0.05; analysed by ANOVA followed by Tukey's post hoc test. RBC = red blood cell count; Hb = hemoglobin; PCV = packed red cell volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume.

Table 4.54: Effect of oral administration of aqueous leaf extract of *Azadiratchta indica* for 28 days on erythrocytes and related parameters in mice

Treatment (mg/kgbw)	Hematological parameters and indices					
	RBC (x10^6/µL)	Hb (g/dL)	PCV (%)	MCH (pg)	MCHC (%)	MCV (fL)
Control	6.40±1.32^{c}	8.90±0.60^{c}	40.28±3.15^{c}	14.47±3.54^{a}	34.24±2.06^{a}	19.82±1.94^{b}
450	8.48±0.84^{c}	10.00±1.00^{c}	42.12±1.46^{bc}	11.81±0.76^{a}	30.70±1.14^{ab}	38.58±2.40^{a}
670	11.62±1.60^{b}	14.06±2.13^{b}	46.02±2.25^{ab}	12.44±3.28^{a}	28.04±3.45^{b}	40.98±2.92^{a}
1000	15.96±3.03^{a}	17.90±1.24^{a}	49.18±3.18^{a}	11.54±2.32^{a}	19.30±2.59^{c}	40.80±3.29^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤0.05; analysed by ANOVA followed by Tukey's post hoc test. RBC = red blood cell count; Hb = hemoglobin; PCV = packed red cell volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume.

Tables 4.55-56 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Lippia javanica* aqueous plant extract for 28 days on erythrocytes and related parameters in mice. Results show that intraperitoneal and oral administration of *L. javanica* to mice for 28 days significantly ($p \leq 0.05$) increased RBC, Hb, MCV and PCV levels and decreased MCH and MCHC levels in a dose dependent manner that picked at 670 mg/kg body weight across the four treatments.

Table 4.55: Effect of intraperitoneal administration of aqueous leaf extract of *Lippia javanica* for 28 days on erythrocytes and related parameters in mice

Treatment (mg/kgbw)	Hematological parameters and indices					
	RBC (x10^6/µL)	**Hb (g/dL)**	**PCV (%)**	**MCH (pg)**	**MCHC (%)**	**MCV (fL)**
Control	9.28±0.91^{c}	7.96±0.78^{c}	43.20±3.29^{b}	8.65±1.26^{a}	35.22±2.04^{a}	27.22±2.38^{c}
450	11.44±1.43^{cb}	13.84±2.38^{b}	39.22±3.12^{b}	12.40±3.30^{a}	32.08±1.49^{a}	39.84±2.90^{b}
670	13.04±1.58^{b}	13.26±2.48^{b}	42.40±1.44^{b}	10.27±2.20^{a}	25.08±3.00^{b}	41.82±2.59^{ba}
1000	19.50±0.68^{a}	17.44±1.25^{a}	49.28±3.12^{a}	8.96±0.87^{a}	20.46±3.38^{b}	44.84±1.92^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤0.05; analysed by ANOVA followed by Tukey's post hoc test. RBC = red blood cell count; Hb = hemoglobin; PCV = packed red cell volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume.

Table 4.56: Effect of oral administration of aqueous leaf extract of *Lippia javanica* for 28 days on erythrocytes and related parameters in mice

Treatment (mg/kgbw)	Hematological parameters and indices					
	RBC (x10^6/µL)	**Hb (g/dL)**	**PCV (%)**	**MCH (pg)**	**MCHC (%)**	**MCV (fL)**
Control	6.40±1.32^{c}	8.90±0.60^{b}	40.28±3.15^{bc}	14.45±3.54^{a}	34.24±2.06^{a}	19.82±1.94^{c}
450	7.76±0.50^{c}	9.98±0.25^{b}	37.20±2.06^{c}	12.90±0.81^{ba}	32.71±1.69^{a}	34.26±1.64^{b}
670	10.00±1.04^{b}	10.66±1.11^{b}	42.13±1.45^{b}	10.73±1.43^{bc}	27.92±3.02^{b}	37.02±1.39^{b}
1000	19.32±0.61^{a}	16.88±1.49^{a}	48.94±3.15^{a}	8.76±0.96^{c}	21.72±2.79^{c}	44.28±2.45^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤0.05; analysed by ANOVA followed by Tukey's post hoc test. RBC = red blood cell count; Hb = hemoglobin; PCV = packed red cell volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume.

Tables 4.57-58 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Ocimum lamiifolium* aqueous plant extract for 28 days on erythrocytes and related parameters in mice.

Results show that intraperitoneal and oral administration of *O. lamiifolium* to mice for 28 days significantly ($p \leq 0.05$) increased RBC, Hb, MCV and PCV levels and decreased MCH and MCHC levels in a dose related manner. Therefore, a treatment dosage of 1000mg/kg of the extract recorded highest values for RBC, Hb, MCV and PCV and lowest values for MCH and MCHC relative to that of the control mice in both oral and intraperitoneal routes.

Table 4.57: Effect of intraperitoneal administration of aqueous leaf extract of *Ocimum lamiifolium* for 28 days on erythrocytes and related parameters in mice

Treatment (mg/kgbw)	Hematological parameters and indices					
	RBC (x10^6/µL)	Hb (g/dL)	PCV (%)	MCH (pg)	MCHC ((%)	MCV (fL)
Control	9.28±0.91^{c}	7.96±0.78^{c}	43.20±3.29^{b}	8.65±1.26^{a}	35.22±2.04^{a}	27.22±2.38^{c}
450	9.55±1.21^{c}	10.46±1.29^{cb}	39.54±3.05^{b}	11.08±1.97^{a}	31.22±1.48^{a}	37.08±1.21^{b}
670	13.01±1.73^{b}	12.88±2.20^{ba}	42.66±1.05^{b}	10.47±2.14^{a}	24.76±3.09^{b}	41.62±3.18^{a}
1000	19.38±1.41^{a}	15.68±1.89^{a}	49.34±3.13^{a}	9.26±0.27^{a}	20.52±2.04^{c}	45.26±0.98^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤0.05; analysed by ANOVA followed by Tukey's post hoc test. RBC = red blood cell count; Hb = hemoglobin; PCV = packed red cell volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume.

Table 4.58: Effect of oral administration of aqueous leaf extract of *Ocimum lamiifolium* for 28 days on erythrocytes and related parameters in mice

Treatment (mg/kgbw)	Hematological parameters and indices					
	RBC (x10^6/µL)	Hb (g/dL)	PCV (%)	MCH (pg)	MCHC (%)	MCV (fL)
Control	6.40±1.32^{c}	8.90±0.60^{b}	40.28±3.15^{bc}	14.47±3.54^{a}	34.24±2.06^{a}	19.82±1.94^{c}
450	7.78±0.25^{c}	10.18±0.38^{b}	37.46±1.82^{c}	12.50±1.31^{a}	32.06±1.66^{a}	34.66±1.98^{b}
670	11.98±1.29^{b}	14.64±1.42^{a}	42.30±1.12^{b}	13.10±2.13^{a}	27.66±2.84^{b}	40.92±4.57^{a}
1000	14.72±1.54^{a}	16.32±1.59^{a}	48.07±3.56^{a}	11.90±1.97^{a}	21.50±2.66^{c}	40.46±1.50^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤0.05; analysed by ANOVA followed by Tukey's post hoc test. RBC = red blood cell count; Hb = hemoglobin; PCV = packed red cell volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume.

Tables 4.59-60 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Persea americana* aqueous plant extract for 28 days on erythrocytes and related parameters in mice. Results show that intraperitoneal and oral administration of *P. americana* to mice for 28 days significantly ($p \leq 0.05$) increased RBC, Hb, MCV and PCV levels and decreased MCH and MCHC levels across the four treatments relative to the control mice.

Table 4.59: Effect of intraperitoneal administration of aqueous leaf extract of *Persea americana* for 28 days on erythrocytes and related parameters in mice

Treatment (mg/kgbw)	Hematological parameters and indices					
	RBC (x10^6/µL)	**Hb (g/dL)**	**PCV (%)**	**MCH (pg)**	**MCHC (%)**	**MCV (fL)**
Control	9.28±0.91^{c}	7.96±0.78^{c}	43.20±3.29^{b}	8.65±1.26^{b}	35.22±2.04^{a}	27.22±2.38^{b}
450	12.40±0.31^{bc}	14.20±0.69^{b}	45.00±2.21^{ab}	13.26±1.84^{a}	32.58±0.82^{a}	39.84±2.90^{a}
670	15.34±2.09^{ab}	17.70±1.60^{a}	48.46±3.32^{ab}	11.54±2.33^{ab}	31.84±1.00^{a}	40.79±2.28^{a}
1000	15.80±2.51^{a}	17.70±1.78^{a}	50.64±3.52^{a}	10.5±1.11^{ab}	21.66±3.06^{b}	43.70±1.28^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤0.05; analysed by ANOVA followed by Tukey's post hoc test. RBC = red blood cell count; Hb = hemoglobin; PCV = packed red cell volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume.

Table 4.60: Effect of oral administration of aqueous leaf extract of *Persea americana* for 28 days on erythrocytes and related parameters in mice

Treatment (mg/kgbw)	Hematological parameters and indices					
	RBC (x10^6/µL)	**Hb (g/dL)**	**PCV (%)**	**MCH (pg)**	**MCHC (%)**	**MCV (fL)**
Control	6.40±1.32^{c}	8.90±0.60^{c}	40.28±3.15^{c}	14.47±3.54^{a}	34.24±2.06^{a}	19.82±1.94^{c}
450	10.66±0.53^{bc}	10.74±0.98^{cb}	41.08±1.71^{bc}	10.95±1.94^{b}	30.26±1.32^{ab}	36.33±1.19^{b}
670	13.10±1.64^{ba}	13.10±1.84^{b}	45.56±2.46^{ab}	9.70±1.66^{a}	27.78±3.40^{b}	41.00±0.95b^{a}
1000	15.38±2.61^{a}	16.72±1.95^{a}	48.36±3.04^{a}	11.26±1.37^{b}	19.12±2.66^{c}	41.56±2.32^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤0.05; analysed by ANOVA followed by Tukey's post hoc test. RBC = red blood cell count; Hb = hemoglobin; PCV = packed red cell volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume.

4.2.4.2 Determination of Leucocytes and related parameters

Tables 4.61-62 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Croton macrostachyus* for 28 days on differential white blood cell count and other related hematological indices in mice. Results show that intraperitoneal and oral administration of *C. macrostachyus* to mice caused a significant ($p \leq 0.05$) dose dependent increase in WBC, LYM, NEU, BAS, PLT and MPV levels across the four treatments. A treatment dosage of 1000mg/kg of the leaf extract recorded highest values for WBC, LYM, NEU, BAS, PLT and MPV relative to that of the control mice in both routes.

Table 4.61: Effect of intraperitoneal administration of aqueous leaf extract of *Croton macrostachyus* for 28 days on platelets, differential white blood cell count and other related hematological indices in mice

Treatment (mg/kgbw)	Platelets, differential white blood cell count and other hemotological indices							
	WBC ($x10^3/\mu L$)	LYM ($x10^3/\mu L$)	MON ($x10^3/\mu L$)	NEU ($x10^3/\mu L$)	EOS ($x10^3/\mu L$)	BAS ($x10^3/\mu L$)	PLT ($x10^3/\mu L$)	MPV (fL)
Control	8.99 ± 0.23^{c}	5.34 ± 0.18^{a}	0.50 ± 0.20^{b}	2.76 ± 0.21^{c}	0.36 ± 0.09^{c}	0.03 ± 0.02^{b}	209.00 ± 14.37^{c}	9.26 ± 0.11^{b}
450	10.54 ± 1.03^{b}	5.90 ± 1.12^{a}	0.69 ± 0.15^{ba}	3.62 ± 0.24^{b}	0.28 ± 0.15^{c}	0.05 ± 0.02^{b}	225.40 ± 24.92^{c}	9.38 ± 0.18^{b}
670	11.66 ± 0.19^{b}	6.06 ± 0.32^{a}	0.66 ± 0.13^{ab}	4.08 ± 0.36^{b}	0.76 ± 0.11^{b}	0.10 ± 0.02^{b}	273.80 ± 13.22^{b}	9.50 ± 0.10^{ba}
1000	14.98 ± 0.75^{a}	6.26 ± 0.27^{a}	0.78 ± 0.08^{a}	5.58 ± 0.40^{a}	1.03 ± 0.13^{a}	0.75 ± 0.60^{a}	320.20 ± 21.06^{a}	10.10 ± 0.64^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. WBC = white blood cell count; NEU = Neutrophils; EOS = eosinophils; BAS = basophils; MON = monocytes; LYM = lymphocytes; PLT = platelets; MPV = mean platelet volume.

Table 4.62: Effect of oral administration of aqueous leaf extract of *Croton macrostachyus* for 28 days on platelets, differential white blood cell count and other related hematological indices in mice

Treatment (mg/kgbw)	Platelets, differential white blood cell count and other hemotological indices							
	WBC ($x10^3/\mu L$)	LYM ($x10^3/\mu L$)	MON ($x10^3/\mu L$)	NEU ($x10^3/\mu L$)	EOS ($x10^3/\mu L$)	BAS ($x10^3/\mu L$)	PLT ($x10^3/\mu L$)	MPV (fL)
Control	8.78 ± 0.54^{d}	5.20 ± 0.49^{b}	0.40 ± 0.12^{b}	2.76 ± 0.30^{b}	0.40 ± 0.16^{a}	0.02 ± 0.09^{c}	187.24 ± 4.94^{c}	8.66 ± 0.34^{b}
450	9.83 ± 0.41^{c}	5.76 ± 0.27^{ba}	0.58 ± 0.15^{ba}	3.06 ± 0.38^{b}	0.40 ± 0.25^{a}	0.03 ± 0.018^{cb}	215.40 ± 28.08^{cb}	9.30 ± 0.38^{a}
670	10.72 ± 0.17^{b}	5.84 ± 0.18^{ba}	0.60 ± 0.16^{ba}	3.90 ± 0.25^{a}	0.34 ± 0.13^{a}	0.05 ± 0.010^{b}	244.00 ± 13.58^{b}	9.58 ± 0.15^{a}
1000	11.91 ± 0.64^{a}	6.16 ± 0.50^{a}	0.74 ± 0.12^{a}	4.34 ± 0.36^{a}	0.58 ± 0.15^{a}	0.10 ± 0.015^{a}	295.60 ± 23.54^{a}	9.74 ± 0.27^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. WBC = white blood cell count; NEU = Neutrophils; EOS = eosinophils; BAS = basophils; MON = monocytes; LYM = lymphocytes; PLT = platelets; MPV = mean platelet volume.

Tables 4.63-64 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Azadiratchta indica* for 28 days on differential white blood cell count and other related hematological indices in mice.

Results show that intraperitoneal and oral administration of *A. indica* to mice caused a significant ($p \leq 0.05$) dose dependent increase in WBC, LYM, MON, NEU, EOS, BAS, PLT and MPV levels across the four treatments. However, the oral administration of the extract did not significantly ($p \leq 0.05$) change the measured eosinophils levels. At 1000mg/kg the extract recorded highest values for WBC, LYM, NEU, BAS, PLT and MPV relative to that of the control mice in both routes.

Table 4.63: Effect of intraperitoneal administration of aqueous leaf extract of *Azadiratchta indica* for 28 days on platelets, differential white blood cell count and other related hematological indices in mice

Treatment (mg/kgbw)	Platelets, differential white blood cell count and other hemotological indices							
	WBC ($x10^3/\mu L$)	LYM ($x10^3/\mu L$)	MON ($x10^3/\mu L$)	NEU ($x10^3/\mu L$)	EOS ($x10^3/\mu L$)	BAS ($x10^3/\mu L$)	PLT ($x10^3/\mu L$)	MPV (fL)
Control	8.99 ± 0.23^{c}	5.34 ± 0.18^{b}	0.50 ± 0.20^{b}	2.76 ± 0.21^{c}	0.36 ± 0.09^{c}	0.03 ± 0.02^{b}	209.00 ± 14.37^{c}	9.26 ± 0.11^{c}
450	11.04 ± 1.19^{b}	5.98 ± 1.08^{ba}	0.76 ± 0.18^{ba}	3.78 ± 0.30^{b}	0.46 ± 0.11^{c}	0.06 ± 0.02^{bc}	234.00 ± 23.58^{c}	9.54 ± 0.18^{bc}
670	12.09 ± 0.23^{b}	6.06 ± 0.30^{ba}	0.71 ± 0.12^{ba}	4.38 ± 0.32^{b}	0.88 ± 0.11^{b}	0.13 ± 0.03^{b}	279.00 ± 11.94^{b}	9.72 ± 0.16^{b}
1000	15.49 ± 0.27^{a}	6.84 ± 0.18^{a}	0.97 ± 0.19^{a}	5.76 ± 0.52^{a}	1.24 ± 0.10^{a}	1.33 ± 0.09^{a}	354.20 ± 18.66^{a}	10.66 ± 0.35^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. WBC = white blood cell count; NEU = Neutrophils; EOS = eosinophils; BAS = basophils; MON = monocytes; LYM = lymphocytes; PLT = platelets; MPV = mean platelet volume.

Table 4.64: Effect of oral administration of aqueous leaf extract of *Azadiratchta indica* for 28 days on platelets, differential white blood cell count and other related hematological indices in mice

Treatment (mg/kgbw)	Platelets, differential white blood cell count and other hemotological indices							
	WBC ($x10^3/\mu L$)	LYM ($x10^3/\mu L$)	MON ($x10^3/\mu L$)	NEU ($x10^3/\mu L$)	EOS ($x10^3/\mu L$)	BAS ($x10^3/\mu L$)	PLT ($x10^3/\mu L$)	MPV (fL)
Control	8.78 ± 0.54^{d}	5.20 ± 0.49^{b}	0.40 ± 0.12^{b}	2.76 ± 0.30^{b}	0.40 ± 0.16^{a}	0.02 ± 0.01^{c}	187.24 ± 4.94^{d}	8.66 ± 0.34^{c}
450	10.20 ± 0.40^{c}	5.86 ± 0.27^{ba}	0.64 ± 0.18^{ba}	3.16 ± 0.38^{b}	0.50 ± 0.25^{a}	0.04 ± 0.02^{b}	225.40 ± 24.92^{c}	9.38 ± 0.18^{bc}
670	11.12 ± 0.18^{b}	5.96 ± 0.19^{a}	0.64 ± 0.16^{ba}	4.00 ± 0.24^{a}	0.46 ± 0.15^{a}	0.06 ± 0.01^{b}	273.80 ± 13.22^{b}	9.50 ± 0.10^{ba}
1000	12.46 ± 0.55^{a}	6.38 ± 0.48^{a}	0.77 ± 0.13^{a}	4.46 ± 0.34^{a}	0.68 ± 0.15^{a}	0.17 ± 0.01^{a}	319.20 ± 23.90^{a}	10.16 ± 0.69^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. WBC = white blood cell count; NEU = Neutrophils; EOS = eosinophils; BAS = basophils; MON = monocytes; LYM = lymphocytes; PLT = platelets; MPV = mean platelet volume.

Table 4.65-66 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Lippia javanica* for 28 days on differential white blood cell count and other related hematological indices in mice. Results show that intraperitoneal and oral administration of *L. javanica* to mice caused a significant ($p \leq 0.05$) dose dependent increase in WBC, LYM, MON, NEU, EOS, BAS, PLT and MPV levels across the four treatments relative to that of the control mice in both oral and intraperitoneal routes.

Table 4.65: Effect of intraperitoneal administration of aqueous leaf extract of *Lippia javanica* for 28 days on platelets, differential white blood cell count and other related hematological indices in mice

Treatment (mg/kgbw)	Platelets, differential white blood cell count and other hemotological indices							
	WBC ($x10^3/\mu L$)	LYM ($x10^3/\mu L$)	MON ($x10^3/\mu L$)	NEU ($x10^3/\mu L$)	EOS ($x10^3/\mu L$)	BAS ($x10^3/\mu L$)	PLT ($x10^3/\mu L$)	MPV (fL)
Control	8.99 ± 0.24^{c}	5.34 ± 0.18^{b}	0.50 ± 0.20^{b}	2.76 ± 0.21^{c}	0.36 ± 0.09^{c}	0.03 ± 0.02^{b}	209.00 ± 14.37^{c}	9.26 ± 0.11^{b}
450	11.03 ± 1.15^{b}	5.89 ± 1.08^{b}	0.87 ± 0.18^{a}	3.94 ± 0.37^{b}	0.52 ± 0.09^{c}	0.08 ± 0.02^{b}	256.40 ± 18.01^{b}	9.44 ± 0.11^{b}
670	12.08 ± 0.23^{b}	6.16 ± 0.32^{ba}	0.72 ± 0.12^{ba}	4.18 ± 0.36^{b}	0.90 ± 0.14^{b}	0.12 ± 0.03^{b}	279.20 ± 9.68^{b}	9.62 ± 0.08^{b}
1000	15.86 ± 0.16^{a}	6.84 ± 0.18^{a}	0.88 ± 0.08^{a}	5.88 ± 0.43^{a}	1.18 ± 0.11^{a}	1.01 ± 0.47^{a}	340.40 ± 23.67^{a}	10.52 ± 0.58^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. WBC = white blood cell count; NEU = Neutrophils; EOS = eosinophils; BAS = basophils; MON = monocytes; LYM = lymphocytes; PLT = platelets; MPV = mean platelet volume.

Table 4.66: Effect of oral administration of aqueous leaf extract of *Lippia javanica* for 28 days on platelets, differential white blood cell count and other related hematological indices in mice

Treatment (mg/kgbw)	Platelets, differential white blood cell count and other hemotological indices							
	WBC ($x10^3/\mu L$)	LYM ($x10^3/\mu L$)	MON ($x10^3/\mu L$)	NEU ($x10^3/\mu L$)	EOS ($x10^3/\mu L$)	BAS ($x10^3/\mu L$)	PLT ($x10^3/\mu L$)	MPV (fL)
Control	8.78 ± 0.54^{c}	5.20 ± 0.49^{b}	0.40 ± 0.12^{b}	2.76 ± 0.29^{c}	0.40 ± 0.15^{bc}	0.02 ± 0.01^{b}	187.24 ± 4.93^{c}	8.66 ± 0.33^{b}
450	10.22 ± 0.13^{b}	5.68 ± 0.15^{ba}	0.48 ± 0.15^{bc}	3.78 ± 0.26^{b}	0.24 ± 0.13^{c}	0.04 ± 0.01^{b}	240.00 ± 12.75^{b}	9.47 ± 0.15^{a}
670	10.95 ± 0.84^{b}	6.09 ± 1.07^{ba}	0.88 ± 0.30^{a}	3.91 ± 0.33^{b}	0.51 ± 0.14^{b}	0.08 ± 0.02^{b}	238.00 ± 15.05^{b}	9.74 ± 0.45^{ab}
1000	14.82 ± 0.44^{a}	6.52 ± 0.19^{a}	0.76 ± 0.11^{ab}	5.72 ± 0.41^{a}	1.06 ± 0.11^{a}	0.76 ± 0.62^{a}	319.20 ± 23.90^{a}	10.16 ± 0.69^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. WBC = white blood cell count; NEU = Neutrophils; EOS = eosinophils; BAS = basophils; MON = monocytes; LYM = lymphocytes; PLT = platelets; MPV = mean platelet volume.

Tables 4.67-68 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Ocimum lamiifolium* for 28 days on differential white blood cell count and other related hematological indices in mice.

Results show that intraperitoneal and oral administration of *O. lamiifolium* to mice for 28 days caused a significant ($p \leq 0.05$) dose dependent increase in WBC, LYM, NEU, BAS, PLT and MPV levels across the four treatments. However, administration of *Lipia javanica* for 28 days in mice did not significantly ($p \leq 0.05$) change levels of monocytes, basophils (intraperitoneally) and eosinophils (orally). A treatment dosage of 1000mg/kg of the extract recorded highest values for WBC, LYM, NEU, PLT and MPV relative to the control mice in both routes.

Table 4.67: Effect of intraperitoneal administration of aqueous leaf extract of *Ocimum lamiifolium* for 28 days on platelets, differential white blood cell count and other related hematological indices in mice

Treatment (mg/kgbw)	Platelets, differential white blood cell count and other hemotological indices							
	WBC ($x10^3/\mu L$)	LYM ($x10^3/\mu L$)	MON ($x10^3/\mu L$)	NEU ($x10^3/\mu L$)	EOS ($x10^3/\mu L$)	BAS ($x10^3/\mu L$)	PLT ($x10^3/\mu L$)	MPV (fL)
Control	8.99 ± 0.24^{d}	5.34 ± 0.18^{b}	0.50 ± 0.20^{a}	2.76 ± 0.21^{c}	0.36 ± 0.09^{c}	0.03 ± 0.02^{a}	209.00 ± 14.37^{c}	9.26 ± 0.11^{a}
450	10.10 ± 0.92^{c}	5.76 ± 1.04^{ba}	0.60 ± 0.14^{a}	3.44 ± 0.17^{b}	0.25 ± 0.11^{c}	0.05 ± 0.01^{a}	218.00 ± 26.09^{c}	9.12 ± 0.22^{a}
670	11.21 ± 0.21^{b}	5.92 ± 0.34^{ba}	0.56 ± 0.13^{a}	3.98 ± 0.36^{b}	0.66 ± 0.11^{b}	0.09 ± 0.02^{a}	269.20 ± 11.73^{b}	9.34 ± 0.11^{a}
1000	14.24 ± 0.51^{a}	6.38 ± 0.16^{a}	0.66 ± 0.11^{a}	5.62 ± 0.41^{a}	1.06 ± 0.12^{a}	0.51 ± 0.58^{a}	310.80 ± 24.00^{a}	9.30 ± 0.30^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. WBC = white blood cell count; NEU = Neutrophils; EOS = eosinophils; BAS = basophils; MON = monocytes; LYM = lymphocytes; PLT = platelets; MPV = mean platelet volume.

Table 4.68: Effect of oral administration of aqueous leaf extract of *Ocimum lamiifolium* for 28 days on platelets, differential white blood cell count and other related hematological indices in mice

Treatment (mg/kgbw)	Platelets, differential white blood cell count and other hemotological indices							
	WBC ($x10^3/\mu L$)	LYM ($x10^3/\mu L$)	MON ($x10^3/\mu L$)	NEU ($x10^3/\mu L$)	EOS ($x10^3/\mu L$)	BAS ($x10^3/\mu L$)	PLT ($x10^3/\mu L$)	MPV (fL)
Control	8.78 ± 0.54^{c}	5.20 ± 0.49^{b}	0.40 ± 0.12^{ba}	2.76 ± 0.30^{b}	0.40 ± 0.16^{a}	0.02 ± 0.01^{b}	187.24±4.94c	8.66 ± 0.34^{b}
450	9.16 ± 0.47^{c}	5.38 ± 0.27^{ba}	0.36 ± 0.11^{b}	2.92 ± 0.37^{b}	0.48 ± 0.30^{a}	0.02 ± 0.02^{b}	209.20 ± 27.37^{cb}	8.96 ± 0.44^{ba}
670	9.83 ± 0.27^{b}	5.52 ± 0.08^{ba}	0.38 ± 0.15^{ba}	3.68 ± 0.29^{a}	0.22 ± 0.04^{a}	0.03 ± 0.01^{b}	244.80 ± 31.90^{b}	9.42 ± 0.36^{a}
1000	11.39 ± 0.72^{a}	5.98 ± 0.51^{a}	0.62 ± 0.13^{a}	4.22 ± 0.38^{a}	0.48 ± 0.15^{a}	0.09 ± 0.02^{a}	286.20 ± 17.38^{a}	9.48 ± 0.19^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. WBC = white blood cell count; NEU = Neutrophils; EOS = eosinophils; BAS = basophils; MON = monocytes; LYM = lymphocytes; PLT = platelets; MPV = mean platelet volume.

Tables 4.69-70 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Persea americana* for 28 days on differential white blood cell count and other related hematological indices in mice.

Results show that intraperitoneal and oral administration of *P. americana* to mice for 28 days caused a significant ($p \leq 0.05$) dose dependent increase in WBC, LYM, MON, NEU, EOS, PLT and MPV levels that picked at 670 mg/kg body weight across the four treatments. Administration of 1000mg/kg of aqueous leaf extracts recorded highest values for WBC, LYM, MON, EOS, NEU, PLT and MPV relative to that of the control mice in both oral and intraperitoneal routes.

Table 4.69: Effect of intraperitoneal administration of aqueous leaf extract of *Persea americana* for 28 days on platelets, differential white blood cell count and other related hematological indices in mice

Treatment (mg/kgbw)	Platelets, differential white blood cell count and other hemotological indices							
	WBC ($x10^3/\mu L$)	LYM ($x10^3/\mu L$)	MON ($x10^3/\mu L$)	NEU ($x10^3/\mu L$)	EOS ($x10^3/\mu L$)	BAS ($x10^3/\mu L$)	PLT ($x10^3/\mu L$)	MPV (fL)
Control	8.99 ± 0.24^{d}	5.34 ± 0.18^{c}	0.50 ± 0.20^{b}	2.76 ± 0.21^{c}	0.36 ± 0.09^{b}	0.03 ± 0.02^{b}	209.00 ± 14.37^{b}	9.26 ± 0.11^{b}
450	10.32 ± 0.21^{c}	5.65 ± 0.23^{cb}	0.47 ± 0.16^{b}	3.94 ± 0.26^{b}	0.37 ± 0.33^{b}	0.05 ± 0.02^{b}	259.80 ± 18.42^{ba}	9.72 ± 0.13^{b}
670	11.84 ± 0.63^{b}	6.16 ± 0.54^{b}	0.71 ± 0.11^{ba}	4.32 ± 0.22^{b}	0.61 ± 0.11^{b}	0.10 ± 0.03^{b}	235.60 ± 134.20^{ba}	9.70 ± 0.24^{b}
1000	15.63 ± 0.17^{a}	6.82 ± 0.29^{a}	0.93 ± 0.21^{a}	5.82 ± 0.69^{a}	1.26 ± 0.09^{a}	1.27 ± 0.15^{a}	348.60 ± 21.02^{a}	10.40 ± 0.56^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. WBC = white blood cell count; NEU = Neutrophils; EOS = eosinophils; BAS = basophils; MON = monocytes; LYM = lymphocytes; PLT = platelets; MPV = mean platelet volume.

Table 4.70: Effect of oral administration of aqueous leaf extract of *Persea americana* for 28 days on platelets, differential white blood cell count and other related hematological indices in mice

Treatment (mg/kgbw)	Platelets, differential white blood cell count and other hemotological indices							
	WBC ($x10^3/\mu L$)	LYM ($x10^3/\mu L$)	MON ($x10^3/\mu L$)	NEU ($x10^3/\mu L$)	EOS ($x10^3/\mu L$)	BAS ($x10^3/\mu L$)	PLT ($x10^3/\mu L$)	MPV (fL)
Control	8.78 ± 0.54^{c}	5.20 ± 0.49^{b}	0.40 ± 0.12^{b}	2.76 ± 0.29^{d}	0.40 ± 0.15^{c}	0.02 ± 0.01^{b}	187.24 ± 4.93^{d}	8.66 ± 0.33^{b}
450	10.45 ± 1.03^{b}	5.84 ± 1.11^{ba}	0.63 ± 0.13^{a}	3.50 ± 0.27^{c}	0.44 ± 0.28^{cb}	0.05 ± 0.02^{b}	219.80 ± 15.21^{c}	9.24 ± 0.18^{a}
670	11.26 ± 0.25^{b}	6.11 ± 0.39^{ba}	0.60 ± 0.13^{ba}	4.22 ± 0.29^{b}	0.71 ± 0.05^{b}	0.10 ± 0.02^{b}	273.40 ± 14.74^{b}	9.34 ± 0.11^{a}
1000	14.23 ± 0.51^{a}	6.43 ± 0.38^{a}	0.68 ± 0.13^{a}	5.57 ± 0.36^{a}	1.16 ± 0.12^{a}	0.69 ± 0.58^{a}	314.60 ± 23.20^{a}	9.42 ± 0.47^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. WBC = white blood cell count; NEU = Neutrophils; EOS = eosinophils; BAS = basophils; MON = monocytes; LYM = lymphocytes; PLT = platelets; MPV = mean platelet volume.

4.2.5 Determination of biochemical parameters

4.2.5.1 Determination of enzyme markers of liver and kidney functions in mice

Table 4.71−4.72 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Croton macrostachyus* aqueous leaf extract for 28 days on enzyme markers of liver and kidney functions in mice.

Results show that intraperitoneally and orally administered plant extracts of *C. macrostachyus* to mice for 28 days indicated a significant ($p \leq 0.05$) dose dependent increase in ALT, AST, ALP, GGT, LDH, CK and α-AMYL enzymes in the four treatments. In addition, the control treatment recorded the lowest measurements for most enzymes. The intraperitoneal and oral administration of the extract did not significantly ($p \leq 0.05$) change AST/ALT levels across the four treatments.

Table 4.71: Effect of intraperitoneal administration of aqueous leaf extract of *Croton macrostachyus* for 28 days on enzyme markers of liver and kidney functions in mice

Treatment (mg/kgbw)	Enzyme activities							
	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	LDH (U/L)	CK (U/L)	α-AMYL (U/L)	AST/ALT
Control	29.40±1.34^{c}	210.00±10.61^{b}	1.96±0.13^{c}	0.11±0.05^{c}	558.20±69.20^{b}	241.20±59.60^{b}	568.20±48.10^{b}	7.48±0.33^{a}
450	31.80±2.17^{c}	225.00±11.11^{ab}	2.12±0.19^{c}	0.22±0.13^{cb}	672.80±132.85^{b}	246.80±21.32^{b}	632.80±91.30^{b}	7.11±0.72^{a}
670	37.20±2.59^{b}	248.60±44.17^{a}	2.96±0.43^{b}	0.50±0.22^{b}	839.00±65.52^{a}	296.40±10.02^{ba}	930.80±53.58^{a}	6.71±1.53^{a}
1000	43.60±3.58^{a}	270.60±37.90^{a}	3.70±0.34^{a}	1.28±0.18^{a}	959.60±43.06^{a}	333.00±14.58^{a}	1003.80±40.44^{a}	6.24±1.00^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤0.05; analysed by ANOVA followed by Tukey's post hoc test. ALT = alanine transaminase; AST = aspartate transaminase; ALP = alkaline phosphatase; GGT = γ-glutamyltransferase; LDH = lactate dehydrogenase; CK = creatine kinase; α-AMYL = α-amylase; AST/ALT = the ratio of the activity of aspartate transaminase to alanine transaminase.

Table 4.72: Effect of oral administration of aqueous leaf extract of *Croton macrostachyus* for 28 days on enzyme markers of liver and kidney functions in mice

Treatment (mg/kgbw)	Enzyme activities							
	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	LDH (U/L)	CK (U/L)	α-AMYL (U/L)	AST/ALT
Control	31.82±1.31^{c}	216.40±18.57^{c}	2.12±0.15^{d}	0.84±0.38^{b}	768.60±48.70^{c}	226.20±21.94^{c}	842.40±39.20^{b}	7.21±0.54^{a}
450	35.00±1.58^{cb}	244.00±5.24^{cb}	2.58±0.15^{c}	0.32±0.19^{c}	824.60±57.47^{c}	267.00±11.36^{cb}	884.00±60.75^{b}	6.98±0.20^{a}
670	39.80±3.19^{b}	269.80±50.97^{ba}	3.30±0.27^{b}	0.88±0.22^{b}	958.80±52.38^{b}	311.80±11.23^{b}	976.40±45.03^{b}	6.80±1.25^{a}
1000	49.20±4.44^{a}	322.20±22.13^{a}	4.20±0.29^{a}	1.62±0.15^{a}	1055.80±40.22^{a}	394.60±42.26^{a}	1188.60±177.28^{a}	6.57±0.52^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤0.05; analysed by ANOVA followed by Tukey's post hoc test. ALT = alanine transaminase; AST = aspartate transaminase; ALP = alkaline phosphatase; GGT = γ-glutamyltransferase; LDH = lactate dehydrogenase; CK = creatine kinase; α-AMYL = α-amylase; AST/ALT = the ratio of the activity of aspartate transaminase to alanine transaminase.

Table 4.73-74 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Azadiratchta indica* aqueous plant extract for 28 days on enzyme markers of liver and kidney functions in mice. Results show that intraperitoneally and orally administered leaf extract of *A. indica* indicated a significant ($p \leq 0.05$) dose dependent increase in ALT, AST, ALP, GGT, LDH, CK and α-AMYL enzymes across the four treatments relative to the control mice. However, the AST/ALT levels across the four treatments were statistically significant ($p \leq 0.05$) in both routes.

Table 4.73: Effect of intraperitoneal administration of aqueous leaf extract of *Azadiratchta indica* for 28 days on enzyme markers of liver and kidney functions in mice

Treatment (mg/kgbw)	Enzyme activities							
	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	LDH (U/L)	CK (U/L)	α-AMYL (U/L)	AST/ALT
Control	29.40±1.34^{c}	210.00±10.61^{b}	1.96±0.13^{c}	0.11±0.05^{c}	558.20±69.20^{b}	241.20±59.60^{b}	568.20±48.10^{b}	7.48±0.33^{a}
450	30.20±1.48^{c}	214.80±11.12^{a}	2.20±0.13^{c}	0.20±0.04^{cb}	652.80±88.95^{b}	248.80±13.77^{b}	612.40±82.97^{b}	7.10±0.50^{a}
670	35.60±1.82^{b}	244.20±35.57^{a}	2.40±0.23^{b}	0.30±0.12^{b}	791.00±64.07^{a}	266.00±11.25^{ba}	832.40±44.20^{a}	6.90±1.65^{a}
1000	40.60±3.21^{a}	259.40±32.78^{a}	3.56±0.30^{a}	1.16±0.17^{a}	944.80±38.44^{a}	326.80±14.48^{a}	922.80±36.21^{a}	6.44±1.10^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤0.05; analysed by ANOVA followed by Tukey's post hoc test. ALT = alanine transaminase; AST = aspartate transaminase; ALP = alkaline phosphatase; GGT = γ-glutamyltransferase; LDH = lactate dehydrogenase; CK = creatine kinase; α-AMYL = α-amylase; AST/ALT = the ratio of the activity of aspartate transaminase to alanine transaminase.

Table 4.74: Effect of oral administration of aqueous leaf extract of *Azadiratchta indica* for 28 days on enzyme markers of liver and kidney functions in mice

Treatment (mg/kgbw)	Enzyme activities							
	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	LDH (U/L)	CK (U/L)	α-AMYL (U/L)	AST/ALT
Control	31.82±1.31^{c}	216.40±18.57^{b}	2.12±0.15^{c}	0.84±0.38^{b}	768.60±48.70^{c}	226.20±21.94^{c}	842.40±39.20^{b}	7.21±0.54^{a}
450	31.80±2.17^{c}	250.60±13.37^{b}	2.50±0.22^{c}	0.30±0.09^{c}	783.80±18.09^{c}	268.00±18.64^{cb}	846.20±23.41^{b}	7.90±0.78^{a}
670	38.60±3.36^{b}	265.20±49.07^{ba}	3.14±0.26^{b}	0.78±0.16^{b}	954.00±49.71^{b}	304.60±10.67^{b}	959.20±46.21^{b}	6.90±1.25^{a}
1000	47.40±4.22^{a}	310.20±17.24^{a}	4.08±0.30^{a}	1.50±0.16^{a}	1053.80±49.38^{a}	391.20±48.18^{a}	1172.80±167.40^{a}	6.57±0.46^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤0.05; analysed by ANOVA followed by Tukey's post hoc test. ALT = alanine transaminase; AST = aspartate transaminase; ALP = alkaline phosphatase; GGT = γ-glutamyltransferase; LDH = lactate dehydrogenase; CK = creatine kinase; α-AMYL = α-amylase; AST/ALT = the ratio of the activity of aspartate transaminase to alanine transaminase.

Tables 4.75-76 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Lippia javanica* aqueous plant extract for 28 days on enzyme markers of liver and kidney functions in mice.

Results show that intraperitoneally and orally administered plant extracts of *Lippia javanica* to mice for 28 days indicated a significant ($p \leq 0.05$) dose dependent increase in ALT, AST, ALP, GGT, LDH, CK and α-AMYL enzymes in the four treatments. However, the AST/ALT levels across the four treatments were statistically significant ($p \leq 0.05$) in both routes. In addition, oral administration of 1000mg/kg of the extract recorded the highest measurements for ALT, LDH, CK and α-AMYL enzymes activities relative to the control mice.

Table 4.75: Effect of intraperitoneal administration of aqueous leaf extract of *Lippia javanica* for 28 days on enzyme markers of liver and kidney functions in mice

Treatment (mg/kgbw)	Enzyme activities							
	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	LDH (U/L)	CK (U/L)	α-AMYL (U/L)	AST/ALT
Control	29.40±1.34^{b}	210.00±10.61^{a}	1.96±0.13^{b}	0.11±0.05^{b}	558.20±69.20^{b}	241.20±59.60^{ab}	568.20±48.10^{b}	7.48±0.33^{a}
450	27.60±1.14^{b}	215.20±9.52^{a}	1.96±0.27^{b}	0.18±0.05^{ba}	638.60±131.49^{b}	228.60±20.80^{b}	615.20±85.10^{b}	7.81±0.48^{a}
670	30.40±2.41^{b}	218.60±8.85^{a}	2.08±0.24^{b}	0.18±0.08^{ba}	649.60±131.87^{ba}	240.20±19.14^{ba}	624.40±88.97^{b}	7.23±0.61^{a}
1000	35.60±2.70^{a}	244.60±53.91^{a}	2.88±0.40^{a}	0.42±0.26^{a}	828.80±61.98^{a}	292.20±9.34^{a}	922.60±50.76^{a}	6.92±1.65^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. ALT = alanine transaminase; AST = aspartate transaminase; ALP = alkaline phosphatase; GGT = γ-glutamyltransferase; LDH = lactate dehydrogenase; CK = creatine kinase; α-AMYL = α-amylase; AST/ALT = the ratio of the activity of aspartate transaminase to alanine transaminase.

Table 4.76: Effect of oral administration of aqueous leaf extract of *Lippia javanica* for 28 days on enzyme markers of liver and kidney functions in mice

Treatment (mg/kgbw)	Enzyme activities							
	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	LDH (U/L)	CK (U/L)	α-AMYL (U/L)	AST/ALT
Control	31.82±1.31^{b}	216.40±18.57^{b}	2.12±0.15^{c}	0.84±0.38^{a}	768.60±48.70^{b}	226.20±21.94^{b}	842.40±39.20^{b}	7.21±0.54^{a}
450	32.20±1.64^{b}	236.40±5.55^{ba}	2.44±0.13^{b}	0.44±0.40^{b}	782.60±63.30^{b}	249.20±14.52^{b}	818.60±50.19^{b}	7.35±0.32^{a}
670	33.80±1.48^{b}	240.00±5.96^{ba}	2.48±0.15^{b}	0.24±0.17^{ab}	807.40±57.33^{b}	254.40±15.57^{b}	834.20±53.58^{b}	7.11±0.27^{a}
1000	40.00±2.92^{a}	270.20±44.60^{a}	3.52±0.18^{a}	0.86±0.26^{a}	966.60±42.40^{a}	310.80±10.57^{a}	977.60±55.30^{a}	7.49±0.50^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. ALT = alanine transaminase; AST = aspartate transaminase; ALP = alkaline phosphatase; GGT = γ-glutamyltransferase; LDH = lactate dehydrogenase; CK = creatine kinase; α-AMYL = α-amylase; AST/ALT = the ratio of the activity of aspartate transaminase to alanine transaminase.

Tables 4.77-78 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Ocimum lamiifolium* aqueous plant extract for 28 days on enzyme markers of liver and kidney functions in mice.

Results show that intraperitoneally and orally administered plant extracts of *Ocimum lamiifolium* to mice for 28 days indicated a significant ($p \leq 0.05$) dose dependent increase in ALT, AST, ALP, GGT, LDH, CK and α-AMYL enzymes that picked at 670mg/kg body weight across the four treatments. In addition, the control treatment recorded the lowest measurements for most enzymes. However, the AST/ALT levels across the four treatments were statistically significant ($p \leq 0.05$) in the oral route.

Table 4.77: Effect of intraperitoneal administration of aqueous leaf extract of *Ocimum lamiifolium* for 28 days on enzyme markers of liver and kidney functions in mice

Treatment (mg/kgbw)	Enzyme activities							
	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	LDH (U/L)	CK (U/L)	α-AMYL (U/L)	AST/ALT
Control	29.40±1.34^{c}	210.00±10.61^{b}	1.96±0.13^{c}	0.11±0.05^{c}	558.20±69.20^{b}	241.20±59.60^{b}	568.20±48.10^{b}	7.48±0.33^{a}
450	31.60±1.14^{c}	228.60±13.79^{ba}	2.24±0.15^{bc}	0.34±1.13^{cb}	678.40±131.76^{b}	251.60±24.21^{b}	643.60±89.44^{b}	7.24±0.44^{a}
670	39.60±3.78^{b}	255.20±38.50^{ba}	2.78±0.51^{b}	0.40±0.21^{b}	844.50±65.10^{a}	300.90±25.80^{ba}	942.20±46.20^{a}	6.63±0.91^{ab}
1000	46.60±3.05^{a}	277.20±34.08^{a}	3.84±0.29^{a}	1.38±0.18^{a}	965.20±48.22^{a}	337.20±13.74^{a}	1010.00±39.26^{a}	5.95±0.61^{b}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤0.05; analysed by ANOVA followed by Tukey's post hoc test. ALT = alanine transaminase; AST = aspartate transaminase; ALP = alkaline phosphatase; GGT = γ-glutamyltransferase; LDH = lactate dehydrogenase; CK = creatine kinase; α-AMYL = α-amylase; AST/ALT = the ratio of the activity of aspartate transaminase to alanine transaminase.

Table 4.78: Effect of oral administration of aqueous leaf extract of *Ocimum lamiifolium* for 28 days on enzyme markers of liver and kidney functions in mice

Treatment (mg/kgbw)	Enzyme activities							
	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	LDH (U/L)	CK (U/L)	α-AMYL (U/L)	AST/ALT
Control	31.82±1.31^{c}	216.40±18.57^{c}	2.12±0.15^{d}	0.84±0.38^{b}	768.60±48.70^{c}	226.20±21.94^{c}	842.40±39.20^{b}	7.21±0.54^{a}
450	35.20±1.30^{c}	249.60±4.10^{cb}	2.68±0.13^{c}	0.38±0.19^{c}	831.20±54.87^{c}	274.20±12.78^{b}	771.60±270.53^{b}	7.10±0.34^{a}
670	41.10±3.58^{b}	279.60±45.97^{ba}	3.52±0.23^{b}	0.96±0.25^{b}	968.32±52.71^{b}	319.30±11.11^{b}	989.20±30.87^{ab}	6.84±1.19^{a}
1000	50.60±3.91^{a}	326.80±22.62^{a}	4.30±0.36^{a}	1.70±0.10^{a}	1062.00±38.05^{a}	400.80±43.81^{a}	1197.40±179.01^{a}	6.46±0.24^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤0.05; analysed by ANOVA followed by Tukey's post hoc test. ALT = alanine transaminase; AST = aspartate transaminase; ALP = alkaline phosphatase; GGT = γ-glutamyltransferase; LDH = lactate dehydrogenase; CK = creatine kinase; α-AMYL = α-amylase; AST/ALT = the ratio of the activity of aspartate transaminase to alanine transaminase.

Tables 4.79-80 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Persea americana* aqueous plant extract for 28 days on enzyme markers of liver and kidney functions in mice. Results show that intraperitoneally and orally administered plant extracts of *P. americana* at 670 and 1000mg/kg body weight indicated a significant ($p \leq 0.05$) increase in ALT, AST, ALP, GGT, LDH, CK and α-AMYL enzymes relative to those of 450mg/kg and control mice. In addition, the control treatment recorded the lowest measurements for most enzymes. However, the AST/ALT levels across the four treatments were statistically significant ($p \leq 0.05$) in the oral route.

Table 4.79: Effect of intraperitoneal administration of aqueous leaf extract of *Persea americana* for 28 days on enzyme markers of liver and kidney functions in mice

Treatment (mg/kgbw)	Enzyme activities							
	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	LDH (U/L)	CK (U/L)	α-AMYL (U/L)	AST/ALT
Control	29.40±1.34^{b}	210.00±10.61^{a}	1.96±0.13^{b}	0.11±0.05^{b}	558.20±69.20^{b}	241.20±59.60^{b}	568.20±48.10^{b}	7.48±0.33^{a}
450	29.70±2.01^{b}	212.40±8.85^{a}	1.90±0.21^{b}	0.12±0.04^{b}	605.40±90.40^{b}	237.00±16.34^{b}	600.20±86.90^{b}	6.45±0.12^{a}
670	37.82±2.66^{a}	248.20±55.40^{a}	3.12±0.42^{a}	0.59±0.25^{a}	858.00±62.80^{a}	307.60±28.80^{a}	922.20±42.10^{a}	6.36±1.55^{a}
1000	38.00±2.55^{a}	253.20±52.80^{a}	2.98±0.33^{a}	0.58±0.22^{a}	850.30±59.25^{a}	298.72±19.29^{ba}	935.20±62.18^{a}	6.70±1.49^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. ALT = alanine transaminase; AST = aspartate transaminase; ALP = alkaline phosphatase; GGT = γ-glutamyltransferase; LDH = lactate dehydrogenase; CK = creatine kinase; α-AMYL = α-amylase; AST/ALT = the ratio of the activity of aspartate transaminase to alanine transaminase.

Table 4.80: Effect of oral administration of aqueous leaf extract of *Persea americana* for 28 days on enzyme markers of liver and kidney functions in mice

Treatment (mg/kgbw)	Enzyme activities							
	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	LDH (U/L)	CK (U/L)	α-AMYL (U/L)	AST/ALT
Control	31.82±1.31^{b}	216.40±18.57^{a}	2.12±0.15^{b}	0.84±0.38^{a}	768.60±48.70^{b}	226.20±21.94^{b}	842.40±39.20^{b}	7.21±0.54^{a}
450	30.60±1.52^{b}	262.60±9.74^{a}	2.40±0.15^{b}	0.20±0.11^{b}	777.20±15.47^{b}	254.460±13.60^{b}	821.80±24.58^{b}	7.00±0.08^{a}
670	35.40±1.80^{a}	245.00±14.30^{a}	3.10±0.20^{a}	0.90±0.20^{a}	874.80±68.20^{a}	313.00±11.45^{a}	950.00±30.90^{a}	6.76±0.56^{a}
1000	39.40±4.10^{a}	268.60±54.90^{a}	3.28±0.28^{a}	0.92±0.24^{a}	933.60±32.40^{a}	321.60±24.20^{a}	955.20±51.30^{a}	6.70±1.20^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. ALT = alanine transaminase; AST = aspartate transaminase; ALP = alkaline phosphatase; GGT = γ-glutamyltransferase; LDH = lactate dehydrogenase; CK = creatine kinase; α-AMYL = α-amylase; AST/ALT = the ratio of the activity of aspartate transaminase to alanine transaminase.

4.2.5.2 Determination of some blood analytes used to assess liver and kidney function in mice.

Tables 4.81-82 shows the effect of intraperitoneal and oral administration 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Croton macrostachyus* plant extract for 28 days on some blood analytes used to assess liver and kidney function in mice. Results show that intraperitoneally and orally administered plant extract of *C. macrostachyus* in mice for 28 days caused a dose dependent significant ($p \leq 0.05$) increase in CREAT, UREA, BUN, and UA blood analytes levels across the four treatments. The control treatment recorded lowest blood analytes compared to plant extracts along the measured biochemical metabolites.

Table 4.81: Effect of intraperitoneal administration of aqueous leaf extract of *Croton macrostachyus* for 28 days on non-enzyme markers of liver and kidney functions in mice

Treatment (mg/kgbw)	Blood analytes (metabolites)			
	CREAT (µM)	UREA (mM)	BUN (mM)	UA (µM)
Control	10.08 ± 0.22^{c}	3.10 ± 0.16^{c}	1.65 ± 0.09^{b}	56.40 ± 6.02^{c}
450	12.00 ± 1.58^{cb}	3.40 ± 0.29^{c}	1.88 ± 0.48^{b}	62.60 ± 13.07^{cb}
670	13.60 ± 1.14^{b}	3.82 ± 0.28^{b}	1.92 ± 0.22^{b}	85.20 ± 24.47^{b}
1000	15.80 ± 0.84^{a}	4.60 ± 0.16^{a}	2.66 ± 0.34^{a}	123.20 ± 9.84^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. CREAT = creatinine; UREA = Urea; BUN = blood urea nitrogen; UA = uric acid.

Table 4.82: Effect of oral administration of aqueous leaf extract of *Croton macrostachyus* for 28 days on non-enzyme markers of liver and kidney functions in mice

Treatment (mg/kgbw)	Blood analytes (metabolites)			
	CREAT (µM)	UREA (mM)	BUN (mM)	UA (µM)
Control	11.70 ± 1.24^{b}	3.02 ± 0.49^{c}	2.27 ± 0.29^{b}	67.40 ± 2.88^{c}
450	13.80 ± 1.92^{b}	3.44 ± 0.11^{c}	2.48 ± 0.23^{b}	78.00 ± 10.10^{c}
670	16.40 ± 1.14^{a}	4.16 ± 0.24^{b}	2.18 ± 0.08^{b}	112.20 ± 9.26^{b}
1000	16.40 ± 1.14^{a}	5.02 ± 0.25^{a}	3.10 ± 0.16^{a}	169.60 ± 19.93^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. CREAT = creatinine; UREA = Urea; BUN = blood urea nitrogen; UA = uric acid.

Tables 4.83-84 shows the effect of intraperitoneal and oral administration 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Azadiratchta indica* plant extract for 28 days on some blood analytes used to assess liver and kidney function in mice.

Results show that intraperitoneally and orally administered plant extract of *A. indica* to mice for 28 days caused a dose dependent significant ($p \leq 0.05$) increase in CREAT, UREA, BUN, and UA (orally) blood analytes levels across the four treatments. However, intraperitoneally, the change in UA values was not statistically significant ($p \leq 0.05$). The control group recorded lowest blood analytes values compared to plant extracts along the measured biochemical metabolites.

Table 4.83: Effect of intraperitoneal administration of aqueous leaf extract of *Azadiratchta indica* for 28 days on non-enzyme markers of liver and kidney functions in mice

Treatment (mg/kgbw)	Blood analytes (metabolites)			
	CREAT (µM)	UREA (mM)	BUN (mM)	UA (µM)
Control	10.08 ± 0.22^{c}	3.10 ± 0.16^{c}	1.65 ± 0.09^{b}	56.40 ± 6.02^{a}
450	10.80 ± 1.30^{cb}	3.24 ± 0.25^{c}	1.74 ± 0.48^{b}	58.20 ± 15.56^{a}
670	12.40 ± 0.89^{b}	3.70 ± 0.28^{b}	1.82 ± 0.22^{b}	84.60 ± 25.77^{a}
1000	14.80 ± 0.84^{a}	4.50 ± 0.16^{a}	2.56 ± 0.34^{a}	98.90 ± 54.71^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. CREAT = creatinine; UREA = Urea; BUN = blood urea nitrogen; UA = uric acid.

Table 4.84: Effect of oral administration of aqueous leaf extract of *Azadiratchta indica* for 28 days on non-enzyme markers of liver and kidney functions in mice

Treatment (mg/kgbw)	Blood analytes (metabolites)			
	CREAT (µM)	UREA (mM)	BUN (mM)	UA (µM)
Control	11.70 ± 1.24^{b}	3.02 ± 0.49^{c}	2.27 ± 0.29^{b}	67.40 ± 2.88^{c}
450	13.20 ± 2.28^{ba}	3.32 ± 0.13^{c}	2.34 ± 0.18^{b}	74.60 ± 7.77^{cb}
670	16.00 ± 0.71^{a}	4.06 ± 0.08^{b}	2.18 ± 0.08^{b}	76.60 ± 10.99^{b}
1000	15.40 ± 1.14^{a}	4.74 ± 0.48^{a}	3.00 ± 0.16^{a}	155.60 ± 33.71^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. CREAT = creatinine; UREA = Urea; BUN = blood urea nitrogen; UA = uric acid.

Tables 4.85-86 shows the effect of intraperitoneal and oral administration 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Lippia javanica* plant extract for 28 days on some blood analytes used to assess liver and kidney function in mice.

Results show that intraperitoneally administered plant extract of *L. javanica* to mice for 28 days caused a dose dependent significant ($p \leq 0.05$) increase in CREAT, and UA blood analytes levels across the four treatments. However, UREA and BUN were not statistically different following intraperitoneal administration of the extract. Orally administered extract did not significantly ($p \leq 0.05$) alter the values of CREATE and BUN across the four treatments.

Table 4.85: Effect of intraperitoneal administration of aqueous leaf extract of *Lippia javanica* for 28 days on enzyme markers of liver and kidney functions in mice

Treatment (mg/kgbw)	Blood analytes (metabolites)			
	CREAT (µM)	**UREA (mM)**	**BUN (mM)**	**UA (µM)**
Control	10.08±0.22^{b}	3.10±0.16^{a}	1.65±0.09^{a}	56.40±6.02^{b}
450	10.60±0.89^{b}	3.18±0.22^{a}	1.68±0.52^{a}	56.40±16.09^{b}
670	11.02±1.40^{b}	3.24±0.27^{a}	1.86±0.47^{a}	72.40±4.83^{ba}
1000	13.10±0.74^{a}	3.59±0.45^{a}	1.85±0.22^{a}	74.00±6.60^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. CREAT = creatinine; UREA = Urea; BUN = blood urea nitrogen; UA = uric acid.

Table 4.86: Effect of oral administration of aqueous leaf extract of *Lippia javanica* for 28 days on non-enzyme markers of liver and kidney functions in mice

Treatment (mg/kgbw)	Blood analytes (metabolites)			
	CREAT (µM)	**UREA (mM)**	**BUN (mM)**	**UA (µM)**
Control	11.70±1.24^{a}	3.02±0.49^{b}	2.27±0.29^{a}	67.40±2.88^{b}
450	12.60±1.67^{a}	3.22±0.13^{b}	2.28±0.16^{a}	70.00±7.78^{b}
670	14.00±5.24^{a}	3.16±0.18^{b}	2.28±0.08^{a}	73.00±6.16^{b}
1000	15.40±1.14^{a}	4.06±0.24^{a}	2.12±0.08^{a}	107.60±12.76^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. CREAT = creatinine; UREA = Urea; BUN = blood urea nitrogen; UA = uric acid.

Tables 4.87-88 shows the effect of intraperitoneal and oral administration 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Ocimum lamiifolium* plant extract for 28 days on some blood analytes used to assess liver and kidney function in mice. Results show that intraperitoneally and orally administered plant extract of *O. lamiifolium* to mice for 28 days caused a dose dependent significant ($p \leq 0.05$) increase in CREAT, UREA, BUN, and UA blood analytes levels across the four treatments. However, the control treatment recorded lowest blood analytes values compared to plant extracts along the measured biochemical metabolites.

Table 4.87: Effect of intraperitoneal administration of aqueous leaf extract of *Ocimum lamiifolium* for 28 days on non-enzyme markers of liver and kidney functions in mice

Treatment (mg/kgbw)	Blood analytes (metabolites)			
	CREAT (µM)	**UREA (mM)**	**BUN (mM)**	**UA (µM)**
Control	10.08±0.22^{b}	3.10±0.16^{c}	1.65±0.09^{b}	56.40±6.02^{c}
450	11.60±1.14^{b}	3.50±0.23^{c}	2.02±0.43^{b}	65.40±14.45^{cb}
670	15.20±1.92^{a}	3.94±0.30^{b}	1.98±0.16^{b}	89.20±20.83^{b}
1000	16.00±0.71^{a}	4.68±0.19^{a}	2.82±0.36^{a}	126.80±9.31^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤0.05; analysed by ANOVA followed by Tukey's post hoc test. CREAT = creatinine; UREA = Urea; BUN = blood urea nitrogen; UA = uric acid.

Table 4.88: Effect of oral administration of aqueous leaf extract of *Ocimum lamiifolium* for 28 days on non-enzyme markers of liver and kidney functions in mice

Treatment (mg/kgbw)	Blood analytes (metabolites)			
	CREAT (µM)	**UREA (mM)**	**BUN (mM)**	**UA (µM)**
Control	11.70±1.24^{a}	3.02±0.49^{a}	2.27±0.29^{a}	67.40±2.88^{a}
450	15.00±2.65^{b}	3.62±0.24^{d}	2.32±0.23^{c}	79.00±7.84^{c}
670	16.60±1.14^{b}	4.22±0.28^{c}	2.72±0.18^{b}	114.20±9.09^{b}
1000	17.20±2.17^{ba}	5.16±0.23^{b}	3.24±0.09^{a}	172.20±20.92^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤0.05; analysed by ANOVA followed by Tukey's post hoc test. CREAT = creatinine; UREA = Urea; BUN = blood urea nitrogen; UA = uric acid.

Tables 4.89-90 shows the effect of intraperitoneal and oral administration 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Persea americana* plant extract for 28 days on some blood analytes used to assess liver and kidney function in mice.

Results show that intraperitoneally and orally administered plant extract of *P. americana* to mice for 28 days caused a dose dependent significant ($p \leq 0.05$) increase in CREAT, UREA and UA blood analytes levels which picked at 670mg/kg body weight across the four treatments. However, the extract did not significantly ($p \leq 0.05$) alter the values of BUN in both routes. The control treatment recorded lowest blood analytes values compared to plant extracts along the measured biochemical metabolites.

Table 4.89: Effect of intraperitoneal administration of aqueous leaf extract of *Persea americana* for 28 days on non-enzyme markers of liver and kidney functions in mice

Treatment (mg/kgbw)	Blood analytes (metabolites)			
	CREAT (µM)	UREA (mM)	BUN (mM)	UA (µM)
Control	10.08 ± 0.22^{c}	3.10 ± 0.16^{b}	1.65 ± 0.09^{a}	56.40 ± 6.02^{b}
450	11.40 ± 1.14^{cb}	3.28 ± 0.08^{b}	1.80 ± 0.23^{a}	55.40 ± 11.72^{b}
670	12.80 ± 0.84^{ba}	3.78 ± 0.33^{a}	1.98 ± 0.16^{a}	68.40 ± 15.90^{ba}
1000	14.60 ± 1.67^{a}	3.94 ± 0.31^{a}	1.94 ± 0.44^{a}	89.20 ± 20.83^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. CREAT = creatinine; UREA = Urea; BUN = blood urea nitrogen; UA = uric acid.

Table 4.90: Effect of oral administration of aqueous leaf extract of *Persea americana* for 28 days on non-enzyme markers of liver and kidney functions in mice

Treatment (mg/kgbw)	Blood analytes (metabolites)			
	CREAT (µM)	UREA (mM)	BUN (mM)	UA (µM)
Control	11.70 ± 1.24^{b}	3.02 ± 0.49^{b}	2.27 ± 0.29^{a}	67.40 ± 2.88^{c}
450	13.80 ± 3.19^{ab}	3.12 ± 0.29^{b}	222 ± 0.13^{a}	72.00 ± 7.17^{c}
670	15.00 ± 1.58^{a}	4.12 ± 0.26^{a}	2.06 ± 0.13^{a}	87.40 ± 13.56^{a}
1000	15.96 ± 1.87^{a}	4.14 ± 0.24^{a}	2.36 ± 0.11^{a}	89.60 ± 6.58^{b}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. CREAT = creatinine; UREA = Urea; BUN = blood urea nitrogen; UA = uric acid.

4.2.5.3 Determination of some lipid profiles and glucose levels in mice.

Tables 4.91-92 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Croton macrostachyus* plant extract for 28 days on some lipid profiles and glucose levels in mice. Results show that intraperitoneal and oral administration of plant extract of *C. macrostachyus* caused a dose dependent significant ($p \leq 0.05$) decrease in T-BIL, D-BIL, I-BIL, TG, TC, HDL-C, LDL-C and glucose levels across the four treatments.

Table 4.91: Effect of intraperitoneal administration of aqueous leaf extract of *Croton macrostachyus* for 28 days on lipid profiles and glucose levels in mice

Treatment (mg/kgbw)	Lipid profiles and glucose levels							
	T-BIL (µM)	D-BIL (µM)	I-BIL (µM)	TG (mM)	TC (mM)	HDL-C (mM)	LDL-C (mM)	GLUC (mM)
Control	11.38±0.24^{a}	5.88±0.22^{a}	5.50±0.20^{a}	0.68±0.08^{a}	1.56±0.14^{ba}	1.38±0.15c	0.18±0.02^{b}	5.50±0.10^{a}
450	7.72±0.20^{b}	4.20±0.19^{b}	3.52±0.13^{b}	0.63±0.04^{a}	2.10±0.20^{a}	1.82±0.19^{a}	0.29±0.03^{a}	4.96±0.09^{b}
670	6.92±0.17^{c}	3.48±0.08^{c}	3.44±0.15^{b}	0.48±0.08^{b}	1.84±0.14^{bc}	1.66±0.15^{ba}	0.18±0.02^{b}	4.24±0.27^{c}
1000	5.78±0.42^{d}	3.04±0.17^{d}	2.74±0.34^{c}	0.36±0.05^{b}	1.67±0.10^{c}	1.54±0.09^{bc}	0.13±0.02^{c}	3.66±0.19^{d}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. T-BIL = total bilirubin; D-BIL = direct bilirubin; I-BIL = indirect bilirubin; TG = triacylglycerols; TC = total cholesterol; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; GLU = glucose.

Table 4.92: Effect of oral administration of aqueous leaf extract of *Croton macrostachyus* for 28 days on lipid profiles and glucose levels in mice

Treatment (mg/kgbw)	Lipid profiles and glucose levels							
	T-BIL (µM)	D-BIL (µM)	I-BIL (µM)	TG (mM)	TC (mM)	HDL-C (mM)	LDL-C (mM)	GLUC (mM)
Control	12.24±0.35^{a}	6.18±0.22^{a}	6.06±0.21^{a}	0.78±0.08^{a}	1.82±0.17^{a}	1.60±0.16^{a}	0.22±0.02^{a}	6.08±0.26^{a}
450	9.36±0.46^{b}	4.50±0.20^{b}	4.86±0.28^{b}	0.68±0.02^{a}	2.82±0.30^{b}	2.53±0.30^{b}	0.29±0.02^{b}	5.16±0.19^{b}
670	7.28±0.26^{c}	3.64±0.11^{c}	3.64±0.21^{c}	0.56±0.04^{b}	1.95±0.08^{b}	1.72±0.08^{b}	0.23±0.01^{b}	4.68±0.13^{c}
1000	6.32±0.11^{d}	3.36±0.11^{c}	2.96±0.19^{d}	0.44±0.10^{b}	1.88±0.11^{b}	1.74±0.09^{b}	0.14±0.03^{c}	4.02±0.19^{d}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. T-BIL = total bilirubin; D-BIL = direct bilirubin; I-BIL = indirect bilirubin; TG = triacylglycerols; TC = total cholesterol; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; GLU = glucose.

Tables 4.93-94 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Azadiratchta indica* plant extract for 28 days on some lipid profiles and glucose levels in mice.

Results show that intraperitoneally and orally administered plant extract of *A. indica* caused a dose dependent significant ($p \leq 0.05$) decrease in T-BIL, D-BIL, I-BIL, TG, TC, HDL-C, LDL-C and glucose levels which picked at 450mg/kg body weight across the four treatments. Therefore, the control treatments recorded highest values for most parameters.

Table 4.93: Effect of intraperitoneal administration of aqueous leaf extract of *Azadiratchta indica* for 28 days on lipid profiles and glucose levels in mice

Treatment (mg/kgbw)	Lipid profiles and glucose levels							
	T-BIL (µM)	D-BIL (µM)	I-BIL (µM)	TG (mM)	TC (mM)	HDL-C (mM)	LDL-C (mM)	GLUC (mM)
Control	11.38±0.24^{a}	5.88±0.22^{a}	5.50±0.20^{a}	0.68±0.08^{a}	1.56±0.14^{b}	1.38±0.15^{b}	0.18±0.02^{b}	5.50±0.10^{a}
450	7.56±0.21^{b}	4.14±0.17^{b}	3.42±0.13^{b}	0.62±0.04^{a}	1.99±0.21^{a}	1.72±0.19^{a}	0.27±0.03^{a}	4.82±0.15^{b}
670	6.34±0.19^{c}	3.12±0.08^{c}	3.22±0.16^{b}	0.42±0.07^{b}	1.59±0.12^{ba}	1.44±0.11^{ba}	0.15±0.02^{b}	4.08±0.26^{c}
1000	5.46±0.38^{d}	2.86±0.24^{d}	2.62±0.33^{c}	0.30±0.07^{c}	1.57±0.15^{b}	1.44±0.15^{b}	0.13±0.01^{c}	3.52±0.13^{d}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. T-BIL = total bilirubin; D-BIL = direct bilirubin; I-BIL = indirect bilirubin; TG = triacylglycerols; TC = total cholesterol; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; GLU = glucose.

Table 4.94: Effect of oral administration of aqueous leaf extract of *Azadiratchta indica* for 28 days on lipid profiles and glucose levels in mice

Treatment (mg/kgbw)	Lipid profiles and glucose levels							
	T-BIL (µM)	D-BIL (µM)	I-BIL (µM)	TG (mM)	TC (mM)	HDL-C (mM)	LDL-C (mM)	GLUC (mM)
Control	12.24±0.35^{a}	6.18±0.22^{a}	6.06±0.21^{a}	0.78±0.08^{a}	1.82±0.17^{a}	1.60±0.16^{b}	0.22±0.02^{b}	6.08±0.26^{a}
450	9.26±0.46^{b}	4.48±0.23^{b}	4.78±0.26^{b}	0.66±0.16^{a}	2.72±0.31^{b}	2.46±0.31^{a}	0.26±0.03^{a}	5.06±0.19^{b}
670	6.34±0.30^{c}	3.12±0.28^{c}	3.22±0.28^{c}	0.54±0.03^{b}	1.70±0.14^{b}	1.50±0.19^{b}	0.22±0.01^{b}	4.78±0.18^{b}
1000	6.06±0.18^{c}	3.04±0.17^{c}	3.02±0.16^{c}	0.45±0.10^{b}	1.68±0.05^{b}	1.56±0.11^{b}	0.13±0.03^{c}	4.04±0.35^{c}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. T-BIL = total bilirubin; D-BIL = direct bilirubin; I-BIL = indirect bilirubin; TG = triacylglycerols; TC = total cholesterol; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; GLU = glucose.

Tables 4.95-96 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Lippia javanica* plant extract for 28 days on some lipid profiles and glucose levels in mice.

Results show that intraperitoneally and orally administered plant extract of *L. javanica* caused a dose dependent significant ($p \leq 0.05$) decrease in T-BIL, D-BIL, I-BIL, TG, TC, HDL-C and LDL-C levels across the four treatments. The values for most parameters following administration at 670 and 1000 mg/kg body weight of the extract were not significantly different. In addition, the intraperitoneal and oral administration of *L. javanica* aqueous plant extract to mice for 28 days demonstrated a significant decline in blood glucose levels across treatments.

Table 4.95: Effect of intraperitoneal administration of aqueous leaf extract of *Lippia javanica* for 28 days on lipid profiles and glucose levels in mice

Treatment (mg/kgbw)	Lipid profiles and glucose levels							
	T-BIL (µM)	D-BIL (µM)	I-BIL (µM)	TG (mM)	TC (mM)	HDL-C (mM)	LDL-C (mM)	GLUC (mM)
Control	11.38±0.24^{a}	5.88±0.22^{a}	5.50±0.20^{a}	0.68±0.08^{a}	1.56±0.14^{ba}	1.38±0.15^{a}	0.18±0.02^{a}	5.50±0.10^{a}
450	6.66±0.23^{b}	3.28±0.13^{b}	3.38±0.16^{b}	0.47±0.07^{b}	1.72±0.15^{a}	1.56±0.15^{a}	0.16±0.02^{a}	4.08±0.26^{b}
670	5.56±0.42^{c}	2.92±0.16^{c}	2.64±0.34^{c}	0.30±0.07^{c}	1.54±0.08^{ba}	1.42±0.08^{a}	0.12±0.11^{b}	3.42±0.13^{c}
1000	5.46±0.42^{c}	2.80±0.19^{c}	2.54±0.34^{c}	0.24±0.05^{c}	1.45±0.11^{b}	1.34±0.11^{a}	0.11±0.01^{b}	3.20±0.16^{c}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. T-BIL = total bilirubin; D-BIL = direct bilirubin; I-BIL = indirect bilirubin; TG = triacylglycerols; TC = total cholesterol; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; GLU = glucose.

Table 4.96: Effect of oral administration of aqueous leaf extract of *Lippia javanica* for 28 days on lipid profiles and glucose levels in mice

Treatment (mg/kgbw)	Lipid profiles and glucose levels							
	T-BIL (µM)	D-BIL (µM)	I-BIL (µM)	TG (mM)	TC (mM)	HDL-C (mM)	LDL-C (mM)	GLUC (mM)
Control	12.24±0.35^{a}	6.180.22^{a}	6.06±0.21^{a}	0.78±0.08^{a}	1.82±0.17^{a}	1.60±0.16^{a}	0.22±0.02^{a}	6.08±0.26^{a}
450	7.04±0.30^{b}	3.54±0.11^{b}	3.50±0.23^{b}	0.53±0.03^{b}	1.86±0.12^{a}	1.64±0.11^{a}	0.22±0.01^{a}	4.54±0.11^{b}
670	6.12±0.11^{c}	3.26±0.11^{c}	2.86±0.19^{c}	0.44±0.10^{cb}	1.70±0.07^{ba}	1.58±0.08^{a}	0.12±0.03^{b}	3.92±0.19^{c}
1000	6.04±0.11^{c}	3.24±0.11^{c}	2.72±0.22^{c}	0.33±0.08^{c}	1.60±0.08^{b}	1.48±0.08^{a}	0.12±0.02^{b}	3.82±0.19^{c}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. T-BIL = total bilirubin; D-BIL = direct bilirubin; I-BIL = indirect bilirubin; TG = triacylglycerols; TC = total cholesterol; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; GLU = glucose.

Tables 4.97-98 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Ocimum lamiifolium* plant extract for 28 days on some lipid profiles and glucose levels in mice.

Results show that intraperitoneally and orally administered plant extract of *O. lamiifolium* to mice for 28 days showed a dose dependent significant ($p \leq 0.05$) decrease in T-BIL, D-BIL, I-BIL, TG, TC, HDL-C, LDL-C and glucose levels across the four treatments. Therefore, the control treatment recorded highest values for most parameters.

Table 4.97: Effect of intraperitoneal administration of aqueous leaf extract of *Ocimum lamiifolium* for 28 days on lipid profiles and glucose levels in mice

Treatment (mg/kgbw)	Lipid profiles and glucose levels							
	T-BIL (µM)	D-BIL (µM)	I-BIL (µM)	TG (mM)	TC (mM)	HDL-C (mM)	LDL-C (mM)	GLUC (mM)
Control	11.38±0.24^{a}	5.88±0.22^{a}	5.50±0.20^{a}	0.68±0.08^{a}	1.56±0.14^{b}	1.38±0.15^{a}	0.18±0.02^{b}	5.50±0.10^{a}
450	7.50±0.28^{b}	4.10±0.16^{b}	3.36±0.18^{b}	0.66±0.07^{a}	2.05±0.17^{a}	1.72±0.32^{a}	0.25±0.03^{a}	5.22±0.28^{a}
670	7.04±0.11^{b}	3.48±0.08^{c}	3.52±0.16^{b}	0.57±0.43^{a}	1.83±0.20^{ba}	1.64±0.21^{a}	0.19±0.02^{b}	4.34±0.27^{b}
1000	5.92±0.48^{c}	3.12±0.22^{d}	2.80±0.40^{c}	0.48±0.82^{a}	1.65±0.07^{b}	1.52±0.08^{a}	0.13±0.01^{c}	3.82±0.15^{c}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. T-BIL = total bilirubin; D-BIL = direct bilirubin; I-BIL = indirect bilirubin; TG = triacylglycerols; TC = total cholesterol; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; GLU = glucose.

Table 4.98: Effect of oral administration of aqueous leaf extract of *Ocimum lamiifolium* for 28 days on lipid profiles and glucose levels in mice

Treatment (mg/kgbw)	Lipid profiles and glucose levels							
	T-BIL (µM)	D-BIL (µM)	I-BIL (µM)	TG (mM)	TC (mM)	HDL-C (mM)	LDL-C (mM)	GLUC (mM)
Control	12.24±0.35^{a}	6.18±0.22^{a}	6.06±0.21^{a}	0.78±0.08^{a}	1.82±0.17^{b}	1.60±0.16^{b}	0.22±0.02^{b}	6.08±0.26^{a}
450	9.50±0.45^{b}	4.58±0.19^{b}	4.92±0.29^{b}	0.69±0.02^{a}	2.89±0.29^{a}	2.53±0.30^{a}	0.29±0.02^{a}	5.32±0.28^{b}
670	6.82±0.20^{c}	3.44±0.33^{c}	3.38±0.15^{c}	0.55±0.02^{b}	1.93±0.13^{b}	1.70±0.16^{b}	0.24±0.03^{ba}	4.78±0.13^{c}
1000	6.54±0.09^{d}	3.48±0.22^{c}	3.06±0.18^{d}	0.46±0.09^{b}	1.92±0.11^{b}	1.78±0.08^{b}	0.14±0.03^{c}	4.06±0.21^{d}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test. T-BIL = total bilirubin; D-BIL = direct bilirubin; I-BIL = indirect bilirubin; TG = triacylglycerols; TC = total cholesterol; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; GLU = glucose.

Tables 4.99-100 shows the effect of intraperitoneal and oral administration of 450mg/kg, 670mg/kg and 1000mg/kg body weight of *Persea americana* plant extract for 28 days on some lipid profiles and glucose levels in mice. Results show that intraperitoneal and oral administration of the plant extract caused a significant ($p \leq 0.05$) decrease in T-BIL, D-BIL, I-BIL, TG, TC, HDL-C, LDL-C and glucose levels relative to the control group. In both routes the extract did not cause a signicant ($p \leq 0.05$) difference for most parameters at 450 and 670mg/kg body weight.

Table 4.99: Effect of intraperitoneal administration of aqueous leaf extract of *Persea americana* for 28 days on lipid profiles and glucose levels in mice

Treatment (mg/kgbw)	Lipid profiles and glucose levels							
	T-BIL (µM)	D-BIL (µM)	I-BIL (µM)	TG (mM)	TC (mM)	HDL-C (mM)	LDL-C (mM)	GLUC (mM)
Control	11.38±0.24^{a}	5.88±0.22^{a}	5.50±0.20^{a}	0.68±0.08^{a}	1.56±0.14^{b}	1.38±0.15^{a}	0.18±0.02^{b}	5.50±0.10^{a}
450	7.30±0.31^{b}	3.18±0.15^{b}	3.20±0.20^{bc}	0.42±0.05^{bc}	1.78±0.15^{ab}	1.48±0.29^{a}	0.23±0.01^{a}	5.04±0.17^{b}
670	7.00±0.37^{b}	3.38±0.30^{b}	3.32±0.41^{b}	0.46±0.14^{b}	1.72±0.39^{a}	1.46±0.08^{a}	0.21±0.02^{b}	4.44±0.34^{c}
1000	5.94±0.23^{c}	2.66±0.31^{c}	3.02±0.33^{c}	0.33±0.09^{c}	1.68±0.11^{ab}	1.48±0.08^{a}	0.14±0.03^{c}	3.14±0.21^{d5}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤0.05; analysed by ANOVA followed by Tukey's post hoc test. T-BIL = total bilirubin; D-BIL = direct bilirubin; I-BIL = indirect bilirubin; TG = triacylglycerols; TC = total cholesterol; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; GLU = glucose.

Table 4.100: Effect of oral administration of aqueous leaf extract of *Persea americana* for 28 days on lipid profiles and glucose levels in mice

Treatment (mg/kgbw)	Lipid profiles and glucose levels							
	T-BIL (µM)	D-BIL (µM)	I-BIL (µM)	TG (mM)	TC (mM)	HDL-C (mM)	LDL-C (mM)	GLUC (mM)
Control	12.24±0.35^{a}	6.18±0.22^{a}	6.06±0.21^{a}	0.78±0.08^{a}	1.82±0.17^{ba}	1.60±0.16^{a}	0.22±0.02^{a}	6.08±0.26^{a}
450	7.30±0.10^{b}	3.70±0.16^{b}	3.60±0.07^{b}	0.57±0.03^{b}	2.01±0.09^{a}	1.74±0.09^{a}	0.24±0.03^{a}	4.78±0.13^{b}
670	7.16±0.18^{b}	3.80±0.14^{b}	3.60±0.14^{b}	0.54±0.03^{cb}	1.95±0.07^{a}	1.82±0.08^{a}	0.23±0.01^{a}	4.86±0.17^{b}
1000	6.12±0.11^{c}	3.26±0.11^{c}	2.86±0.19^{c}	0.44±0.10^{c}	1.70±0.07^{b}	1.58±0.08^{a}	0.12±0.03^{b}	3.92±0.19^{c}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤0.05; analysed by ANOVA followed by Tukey's post hoc test. T-BIL = total bilirubin; D-BIL = direct bilirubin; I-BIL = indirect bilirubin; TG = triacylglycerols; TC = total cholesterol; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; GLU = glucose.

4.2.6 Qualitative phytochemical screening

Table 4.101 shows qualitative phytochemical composition of aqueous extracts of the five studied medicinal plants. Results show that *C. macrostachyus, A. indica*, and *P. americana* contained alkaloids, sterols, terpenoids, flavonoids, tannins, saponins, free and bound anthraquinones. In fact, *L. javanica* and *O. lamiifolium* did not contain free and bound anthroquinones but indicated the presence of alkaloids, sterols, terpenoids, flavonoids, tannins and saponins.

Table 4.101: Phytochemical composition of the aqueous leaf extracts of the five studied plants

Phytochemicals	Plant Part	Alkaloids	Sterols	Terpenoids	Saponins	Flavanoids	Tannins	Anthraquinones	
								Free	Bound
C. macrostachyus	Leaves	+	+	+	+	+	+	+	+
A. indica	Leaves	+	+	+	+	+	+	+	+
L. javanica	Leaves	+	+	+	+	+	+	-	-
O. lamiifolium	Leaves	+	+	+	+	+	+	-	-
P. Americana	Leaves	+	+	+	+	+	+	+	+

4.2.7 Trace metal analysis

Tables 4.102, 4.103 and 4.104 shows the trace element composition of the five plant extracts traditionally used in the management of diabetes mellitus. Results show that *C. macrostachyus* contained Mg, K, Ca, Mn, Fe, Zn, Br, Rb, Cr, Ti, Cu, V, Cl and Pb; *A. indica* contained Mg, Cr, K, Ca, Ti, Mn, Fe, Cu, Zn, Br, Rb, V, Cl and Pb; *L. javanica* contained Mg, K, Ca, Mn, Fe, Zn, Br, Rb, Ti, V, Cr, Cl and Cu; *O. lamiifolium* contained Mg, K, Ca, Ti, Mn, Fe, Zn, Br, Rb, Cl, Cu and Pb; and *P. americana* contained Mg, K, Ca, Mn, Fe, Zn, Br, Rb, Cr, Ti, Cu, V, Cl and Pb. A dose related increase in the values of mineral elements was also indicated by all the aqueous plant extracts. The five aqueous plant extracts provided Mg, Ca, Pb Cr, Ti, Cu, V, Br and Rb levels above the recommended daily allowance while of Fe, K, Mn, Cl and Zn were below the recommended daily allowance for mice as shown in the Tables 4.102, 4.103 and 4.104.

Table 4.102: Mineral levels (µg/g) and their quantity in 450mg/kg aqueous plant extracts administered to each mouse per day (µg/day)

	PLANTS EXTRACTS					
MINERAL	***Croton macrostachyus***	***Azadiratchta indica***	***Lippia javanica***	***Ocimum lamiifolium***	***Persea americana***	**RDA of mouse (µg/day)**
Mg*	206.5	213	156.5	214.75	204.5	0.0493
	2.137	**2.2045**	**1.6198**	**2.2227**	**2.1166**	
Fe	24.90±0.80	111±2	246±27	63.10±3.00	158±2	2.629
	0.2577	**1.1489**	**2.5461**	**0.6531**	**1.6353**	
K	12737±255	48596±1815	34691±479	7131±136	55270±1253	657.143
	131.828	**502.9695**	**359.0505**	**73.8059**	**572.0445**	
Ca	2148±61	1095±44	9368±690	36788±295	21022±167	0.329
	22.2318	**11.333**	**96.9588**	**380.754**	**217.5778**	
Mn	15.3±0.30	124±1	58.00±1.30	70.30±0.90	269±3	0.756
	0.15836	**1.2834**	**0.6003**	**0.72765**	**2.784**	
Zn	11.40±0.20	62.50±1.80	37.10±0.80	17.20±0.42	88.30±0.80	3.614
	0.11799	**0.6469**	**0.38399**	**0.17802**	**0.9139**	
Pb	< 50	< 50	< 50	1.33±0.45	< 50	0.0092
	< 0.5175	**< 0.5175**	**< 0.5175**	**0.01377**	**< 0.5175**	
Cr*	< 0.2	< 0.2	< 0.2	0	< 0.2	3.286×10^{-6}
	< 0.00207	**< 0.00207**	**< 0.00207**	**0**	**< 0.00207**	
Ti	1.07±0.11	2.07±0.33	9.10±0.62	30.30±1.30	2.72±0.19	4.929×10^{-6}
	0.011075	**0.02142**	**0.09419**	**0.3136**	**0.02815**	
Cu	7.64±0.10	0.58±0.04	3.54±0.20	6.27±0.64	3.42±0.15	1.051×10^{-4}
	0.079074	**0.006003**	**0.036639**	**0.06489**	**0.035397**	
V*	3.60±0.84	< 0.2	< 0.2	0	17.60±1.20	2.103×10^{-5}
	0.03726	**< 0.00207**	**< 0.00207**	**0**	**0.18216**	
Br	177±18	91.20±2.50	97.90±4.10	26.10±0.90	19.20±0.70	2.629×10^{-5}
	1.83195	**0.42476**	**1.0133**	**0.27014**	**0.20493**	
Rb	15.10±0.20	61.60±2.20	18.30±1.30	14.40±0.20	53.60±0.60	1.643×10^{-6}
	0.15629	**0.6376**	**0.1894**	**0.14904**	**0.55476**	
CI	7407±227	5922±365	1030±27	1796±77	4795±109	755.714
	76.66	**61.293**	**10.66**	**18.309**	**49.628**	

Results are expressed as Mean± standard deviation (SD). <means below the limit of detection of TXRF/AAS. Values of trace elements with asterics (*) as a superscript was determined using the AAS. The first row of each mineral indicates mineral levels in the leaf extracts (µg/g) while the second row (in bold) indicates daily mineral administered (µg/day)

Table 4.103: Mineral levels (µg/g) and their quantity in 670mg/kg aqueous plant extracts administered to each mouse per day (µg/day)

	PLANTS EXTRACTS					
MINERAL	***Croton macrostachyus***	***Azadiratchta indica***	***Lippia javanica***	***Ocimum lamiifolium***	***Persea americana***	**RDA (µg/day)**
Mg*	206.5	213	156.5	214.75	204.5	0.0493
	3.1821	**3.2823**	**2.4117**	**3.3093**	**3.1535**	
Fe	24.90±0.80	111±2	246±27	63.10±3.00	158±2	2.629
	0.3837	**1.7105**	**3.7909**	**0.9724**	**2.4348**	
K	12737±255	48596±1815	34691±479	7131±136	55270±1253	657.143
	196.2772	**748.866**	**534.586**	**109.889**	**851.7107**	
Ca	2148±61	1095±44	9368±690	36788±295	21022±167	0.329
	33.1007	**16.874**	**144.361**	**566.9004**	**323.9490**	
Mn	15.3±0.30	124±1	58.00±1.30	70.30±0.90	269±3	0.756
	0.2358	**1.9108**	**0.8938**	**1.0834**	**4.1453**	
Zn	11.40±0.20	62.50±1.80	37.10±0.80	17.20±0.42	88.30±0.80	3.614
	0.1757	**0.9631**	**0.5717**	**0.2650**	**1.3607**	
Pb	< 50	< 50	< 50	1.33±0.45	< 50	0.0092
	< 0.7705	**< 0.7705**	**< 0.7705**	**0.0205**	**< 0.7705**	
Cr*	< 0.2	< 0.2	< 0.2	0	< 0.2	3.286×10^{-6}
	< 0.00308	**< 0.00308**	**< 0.00308**	**0**	**< 0.00308**	
Ti	1.07±0.11	2.07±0.33	9.10±0.62	30.30±1.30	2.72±0.19	4.929×10^{-6}
	0.01649	**0.0319**	**0.1402**	**0.467**	**0.04192**	
Cu	7.64±0.10	0.58±0.04	3.54±0.20	6.27±0.64	3.42±0.15	1.051×10^{-4}
	0.1177	**0.00894**	**0.05455**	**0.8142**	**0.0527**	
V*	3.60±0.84	< 0.2	< 0.2	0	17.60±1.20	2.103×10^{-5}
	0.05548	**< 0.00308**	**< 0.00308**	**0**	**0.2712**	
Br	177±18	91.20±2.50	97.90±4.10	26.10±0.90	19.20±0.70	2.629×10^{-5}
	2.728	**1.4054**	**1.5086**	**0.4022**	**0.30512**	
Rb	15.10±0.20	61.60±2.20	18.30±1.30	14.40±0.20	53.60±0.60	1.643×10^{-6}
	0.0.2327	**0.9493**	**0.2820**	**0.2219**	**0.826**	
CI	7407±227	5922±365	1030±27	1796±77	4795±109	755.714
	114.14	**91.258**	**15.87**	**27.260**	**73.891**	

Results are expressed as Mean± standard deviation (SD). <means below the limit of detection of TXRF/AAS. Values of trace elements with asterics (*) as a superscript was determined using the AAS. The first row of each mineral indicates mineral levels in the leaf extracts (µg/g) while the second row (in bold) indicates daily mineral administered (µg/day)

Table 4.104: Mineral levels (µg/g) and their quantity in 1000mg/kg aqueous plant extracts administered to each mouse per day (µg/day)

	PLANTS EXTRACTS					
MINERAL	***Croton macrostachyus***	***Azadiratchta indica***	***Lippia javanica***	***Ocimum lamiifolium***	***Persea americana***	**RDA (µg/day)**
Mg*	206.5	213	156.5	214.75	204.5	0.0493
	4.7495	**4.899**	**3.5995**	**4.9393**	**4.7035**	
Fe	24.90±0.80	111±2	246±27	63.10±3.00	158±2	2.629
	0.5727	**2.553**	**5.658**	**1.4513**	**3.634**	
K	12737±255	48596±1815	34691±479	7131±136	55270±1253	657.143
	292.951	**1117.71**	**797.89**	**164.013**	**1271.21**	
Ca	2148±61	1095±44	9368±690	36788±295	21022±167	0.329
	49.404	**25.185**	**215.464**	**846.12**	**483.506**	
Mn	15.3±0.30	124±1	58.00±1.30	70.30±0.90	269±3	0.756
	0.3519	**2.852**	**1.334**	**1.617**	**6.187**	
Zn	11.40±0.20	62.50±1.80	37.10±0.80	17.20±0.42	88.30±0.80	3.614
	0.2622	**1.4375**	**0.8533**	**0.3956**	**2.0309**	
Pb	< 50	< 50	< 50	1.33±0.45	< 50	0.0092
	< 1.15	**< 1.15**	**< 1.15**	**0.03059**	**< 1.15**	
Cr*	< 0.2	< 0.2	< 0.2	0	< 0.2	3.286×10^{-6}
	< 0.0046	**< 0.0046**	**< 0.0046**	**0**	**< 0.0046**	
Ti	1.07±0.11	2.07±0.33	9.10±0.62	30.30±1.30	2.72±0.19	4.929×10^{-6}
	0.02461	**0.04761**	**0.2093**	**0.6969**	**0.06256**	
Cu	7.64±0.10	0.58±0.04	3.54±0.20	6.27±0.64	3.42±0.15	1.051×10^{-4}
	0.17572	**0.01334**	**0.08142**	**0.14421**	**0.07866**	
V*	3.60±0.84	< 0.2	< 0.2	0	17.60±1.20	2.103×10^{-5}
	0.0828	**< 0.0046**	**< 0.0046**	**0**	**0.4048**	
Br	177±18	91.20±2.50	97.90±4.10	26.10±0.90	19.20±0.70	2.629×10^{-5}
	4.071	**2.0976**	**2.2517**	**0.6003**	**0.4554**	
Rb	15.10±0.20	61.60±2.20	18.30±1.30	14.40±0.20	53.60±0.60	1.643×10^{-6}
	0.3473	**1.4168**	**0.4209**	**0.3312**	**1.2328**	
CI	7407±227	5922±365	1030±27	1796±77	4795±109	755.714
	170.361	**136.206**	**23.69**	**40.687**	**110.285**	

Results are expressed as Mean± standard deviation (SD). <means below the limit of detection of TXRF/AAS. Values of trace elements with asterics (*) as a superscript was determined using the AAS. The first row of each mineral indicates mineral levels in the leaf extracts (µg/g) while the second row (in bold) indicates daily mineral administered (µg/day)

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

The alloxan-induced diabetic mice had a four to five fold elevation in blood glucose levels (5mg/dl to 25mg/dl) relative to the normal control mice. Studies show that chemical induction of diabetes by intraperitoneal administration of a diabetogenic agent, alloxan monohydrate induces Type I diabetes in experimental animals (Viana *et al.,* 2004; Etuk, 2010). Alloxan monohydrate causes selective necrosis of pancreatic beta-cells of Langerhans (Iranloye *et al.,* 2011). This therefore, affects endogenous insulin synthesis and release making it biologically unavailable or insufficient and thus results in hyperglycemia (Nastaran, 2011). The intraperitonial and oral administration of the aqueous leaf extracts of *Croton macrostachyus, Azadiratchta indica, Lippia javanica, Ocimum lamiifolium* and *Persea americana* demonstrated blood glucose lowering effect in mice indicating that they contained hypoglycemic constituents.

The possible mechanism of action for hypoglycemic effect of these plant extracts was either through increased utilization of glucose by peripheral tissues such as the muscle, fat and liver cells via activation of the insulin receptors or direct stimulation of remnant β-cells of islet of Langerhans to secrete insulin (Ayodhya *et al.,* 2010). Increased serum insulin consequently resulted in reduced blood sugar by increased facilitated uptake of glucose by peripheral tissues mediated by GLUT-4, an insulin dependent glucose transporter (Obatomi *et al.,* 1994; Shafighi *et al.,* 2013). The plant extracts might have also conferred antioxidative protective mechanism of the

pancreatic beta cells. The plants' antihyperglycemic action might also be attributed to interference on absorption of dietary carbohydrates as well as disaccharides in small intestines of mice by slowing gastric motility and emptying (Ortiz-Andrade *et al.,* 2007). It can also be as a result of regeneration of β-cells (Esmaeili and Yazdanparas, 2004; Sharma *et al.,* 2006), and/or restored insulin sensitivity (Yolanda *et al.,* 2006).

The blood glucose lowering effect of these plants was similar to that reported of other plants investigated by early researches. The leaves and bulb of the plant *Allium sativum* (garlic) demonstrated antidiabetic activity by increasing either the pancreatic secretion of insulin from the β cells or the release of bound insulin (Syed *et al.,* 2013). Phytoconstituents present in garlic also showed antioxidative property evidenced by scavenging of reactive oxygen species and increasing cellular antioxidant enzymes: superoxide dismutase, catalase, and glutathione peroxidase (Syed *et al.,* 2013). Murugi *et al.* (2012) observed tha**t** aqueous leaf extracts of *Caesalpinia volkensii* showed hypoglycemic activity in alloxan-induced diabetic mice through administration of 50,100 and 150mg/kg body weight**.**

Leaf extract of *Ocimum sanctum* has been reported to stimulate the physiological pathways of insulin secretion, reduce the serum level of cortisol and glucose in male mice thus exhibing its antiperoxidative effect (Rani *et al.,* 2013). *Momordica charantia* has demonstrated antidiabetic activities and lipid-lowering properties due to its ability to repair damaged β-cells thereby stimulating insulin levels and also improve sensitivity/signaling of insulin (Syed *et al.,* 2013). It's also reported to inhibit

absorption of glucose by inhibiting glucosidase and suppressing the activity of disaccharidases in the intestine (Syed *et al.,* 2013).

The higher antidiabetic effect demonstrated by the antihyperglycemic plant extracts administered via intraperitoneal route relative to the oral route could be due to the fact that in the oral route the constituents may have been transported more slowly across the intestinal wall while by intraperitoneal route the active compounds reached the systemic circulation faster (Mukundi *et al.,* 2015; Abdirahman *et al.,* 2015). Through the oral route, the extracts may have been degraded or inactivated by digestive enzymes such as amylase and lipases in gastrointestinal mucosa (Ortiz-Andrade *et al.,* 2007). The route of administration therefore brings the difference in activity atttributable to the much higher bioavailability of the extract when administered intraperitoneally (three-four more times bioavailable than oral route) (Meezan *et al.,* 2005).

The observed dose independent hypoglycemic action of the plant extracts may reflect uptake of the active constituents through saturable active transport, it may also suggest maximum hypoglycemic activity at the lowest dose used (25mg/kg body weight) (Shafighi *et al.*, 2013). This study concurs with other findings of different Lantana species which also demonstrated anti-diabetic effects (Okyar *et al*., 2001).

The plant extracts also showed insulinemimetic activity and at times worked better than conventional drugs in both routes, these may have been due to increased uptake

of glucose by peripheral tissues mediated by GLUT-4 or the extracts might have been easily absorbed in the intraperitoneal cavity and gastro intestinal mucosa. Besides, there could be no compound in the extracts that can act as a pro-drug thus the active principles in the extracts did not require biotransformation so as to become antihyperglycemic (Kaliwal *et al.*, 2012).

A comparison of the difference in antidiabetic effect of the five aqueous plant extracts in mice models at various dosages indicated that *A. indica* and *O. lamiifolium* demonstrated the highest antidiabetic effect as compared to other plant extracts with an optimal dose occurring between the third and the seventh hour where the blood sugar reduction rate was between 45% and 75%. This implies that the concentration of an antidiabetic compound is influenced by the difference in soil composition and variation in environmental stresses where the plant grows. Bioactive compounds are normally the secondary products produced by plants to help them survive in different environmental stresses (Nyamai *et al.*, 2016). This therefore, indicates that *A. indica* and *O. lamiifolium* contained a much higher content of phytoconstituents that confers hypoglycemic activity as opposed to other extracts as a result of environmental stresses in the ecological niche where these plants grew.

The recovery phase observed in the twenty fourth hour by all extracts in both routes help identify frequency of dosing of the antidiabetic drugs. The return to diabetic states in this hour may be due to the reduction of antidiabetic activity of the extracts as

a result of fast hepatic metabolism and renal clearance or the extracts having a short half-life (Mukundi *et al.,* 2015).

The antihyperglcemic effect of the aqueous plant extracts might also be attributed to the presence of various phytoconstituents including: tannins, flavanoids, saponins, alkaloids, terpenoids, sterols, free and bound anthraquinones that have been associated with antidiabetic activity (Elliot *et al.,* 2000).

The antidiabetic effect of *C. macrostachyus, A. indica, L. javanica, O. lamiifolium* and *P. americana* could have been due to the observed presence of flavonoids. . As reported by Glauce *et al*. (2004), 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). Epicatechin and its active principles have demonstrated *in vitro* that they facilitate insulin release through conversion of pro-insulin to insulin (Modak *et al.,* 2007). It has been shown that the flavonoid fraction from *Pterocarpus marsupiu* could cause pancreatic beta cell regranulation. Flavonoid glycosides of *Psidium guajava* such as strictinin, isostrictinin and pedunculagin have been used in clinical treatment of diabetes due to improved sensitivity of insulin (Chauhan, 2010). At 50-150 mg/kg flavonoids isolated from leaf of *Ipomoea batatas*, reduced blood glucose level and lipid parameters in alloxan induced diabetic mice (Li *et al.,* 1999).

The alkaloids present in the aqueous leaf extract of these plants have also been reported to have antihyperglycemic activity. Alkaloids berberine and tetrandine have been reported to demonstrate antioxidant activity responsible for various biological activities associated with this plant including antidiabetic activity (Shukla *et al*., 2012). Alkaloid fraction from *C. decidua* showed hypoglycemic potential in mice (Sharma, 2010). The alkaloids l-ephedrine of *Ephedra distachya* herbs have shown hypoglycemic effect in diabetic mice due to restoration and regeneration of atrophied pancreatic islets that induces the secretion of insulin (Elliot *et al*., 2010).

The saponins present in the aqueous leaf extract of these plants have also been reported to have antihyperglycemic activity. For instance, ginseng and its saponins have been shown to lower blood glucose in alloxan-treated, genetically diabetic, and normal mice (Kimura *et al.*, 1985). Saponins have also been shown to have hypoglycemic activity as demonstrated by Sui *et al*. (1994) where saponins isolated from the leaves of *Acanthopanax senticosus* injected into mice (100, 200 mg/kg, 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* tested on normalglycemic rabbits at a dose of 50 mg/kg showed that the saponins-component reduced glycemia after 1, 2, 3 and 6 hour. Graded doses of saponins extract (10, 15 and 20 mg/kg) caused a marked hypoglycemic effect in alloxan-induced diabetic rabbits (Abdel-Hassan *et al*., 2000). In elderly patients with hyperglycemia, saponins were shown to have reduced serum glucose (Chen and Zhang, 1987).

The aqueous leaf extract of *C. macrostachyus, A. indica, L. javanica, O. lamiifolium* and *P. americana*, contained tannins that are known to contain antidiabetic activity. Broadhurst *et al.* (1997) demonstrated that tannin epigllo-catechin-3-gallate exhibits hypoglycemic activity. Tannins are polyphenols from multiple species classified into two broad groups; hydrolysable tannins and condensed tannins. In clinical terms, all forms of tannins may participate in the management of glucose level in blood. Tannin has been shown to stimulate the receptor cells to utilize carbohydrate (Kumari *et al.,* 2014a).

The aqueous leaf extracts of *C. macrostachyus, A. indica, L. javanica, O. lamiifolium* and *P. americana*, contains terpenoids which are heart-friendly antidiabetic phytoconstituents. Terpenoids have shown to reduce diastolic blood pressure and lower the sugar level in blood in hypertensive and diabetic patients respectively (Hawkins and Ehrlich, 2006). Terpenoids also improves the skin tone, increases the concentration of antioxidants in wounds, and restore inflammed tissues by increasing blood supply (Piero *et al.*, 2015a). Terpenoids also improve lung function. The leaves and seeds of *E. officinalis* are used in the treatment of diabetes due to the presence of terpenoids (Treadway, 1994).

The aqueous leaf extracts of *C. macrostachyus, A. indica,* and *P. americana*, contained free and bound anthraquinones which have previously been reported to have the hypoglycemic activities. The antraquionone in the plant extracts of *Polygonum multiflorum* have been used in the management of peripheral neuropathy, a

complication associated with diabetes mellitus (Broadhurst, 1997). The plant extracts contained steroids that make them a good source of steroidal compounds which are potent precursors for the synthesis of sex hormones (Piero *et al.,* 2015b).

In the recent researches a relationship has been observed between diabetes mellitus and trace elements. Insulin action has been found to be potentiated by some trace elements like zinc, magnesium, manganese, chromium, molybdenum vanadium and selenium (Ngugi *et al.,* 2012b). The possible mechanisms of enhancement of insulin action by the trace elements include activation of insulin receptor sites, acting as cofactors for various enzyme involved in glucose metabolism, stimulating insulin sensitivity and serving as antioxidants for preventing tissue peroxidation (Siddiqui *et al.,* 2014). Consequently, it has been demostrated that the decreased levels of trace elements could cause disturbances in glucose transport across cell membrane leading to insufficient formation and secretion of insulin by pancreas thereby compromising the antioxidant defense mechanisms (Siddiqui *et al.,* 2014).

In this study, analysis of the five plants revealed the presence of Mg, K, Ca, Mn, Fe, Zn, Br, Rb, Cr, Ti, Cu, V, Cl and Pb. Magnesium is an essential mineral ion involved in multiple levels in insulin's secretion, its binding and its activity; it is a cofactor in various enzyme pathways involved in glucose oxidation, and it also modulates glucose transport across cell membranes (Siddiqui *et al.,* 2014). Magnesium increases insulin secretion and/or improves insulin sensitivity and peripheral glucose uptake (Salmonowicz *et al.,* 2014). It has no effect on hepatic glucose output and non-

oxidative glucose disposal. Consumption of $MgSO_4$ in diabetic rats was found to reverse the high blood glucose level (Parvizi *et al.,* 2014). However, $MgSO_4$ couldn't return it to the control levels in the treated diabetic rats. Solaimani *et al.* (2014) suggested that blood glucose levels can be decreased by Mg via increasing GLUT4 mRNA expression in diabetic rats independent to insulin secretion.

Manganese is essential for human health. It functions as a key constituent of metallo-enzymes activator in cellular biochemical reactions (Siddiqui *et al.,* 2014). It activates an antioxidant enzyme manganese superoxide dismutase (MnSOD) that protects the cell membranes and tissues from disruption and degeneration. It helps the body to catabolize lipids, carbohydrates, and proteins and assist in energy production (George *et al.,* 2004). It is also involved in the modulation of glucose transport across cell membranes (Mooradian and Morley, 1987; O'Connell, 2001). Manganese deficiency causes impaired glucose tolerance, impaired growth, impaired reproductive function, skeletal abnormalities, and altered carbohydrate and lipid metabolism (Salmonowicz *et al.,* 2014). Manganese supplements have been shown to reverse the impaired glucose utilization induced by manganese deficiency in guinea pigs (Rajendra *et al.,* 2007).

Vanadium affects carbohydrate metabolism including glucose transport, glycolysis, glucose oxidation, and glycogen synthesis (Siddiqui *et al.,* 2014). At a dose of 100 mg/day vanadyl sulfate improves insulin sensitivity (Siddiqui *et al.,* 2014). Its possible mechanism of action in glycemic control is thought to be primarily insulinomimetic

with up regulation of insulin receptors. It facilitates glucose uptake and metabolism and enhances insulin sensitivity in animal models and clinically, it has been shown to enhance glucose oxidation and glycogen synthesis, and it modulates hepatic glucose output (O'Connell, 2001).

Zinc has been shown to be involved in virtually all aspects of insulin metabolism: synthesis, secretion and utilization ((Siddiqui *et al.,* 2014). It is required as a cofactor for the function of intracellular enzymes that may be involved in protein, lipid, and glucose metabolism (Siddiqui *et al.,* 2014). Zinc 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 *et al*., 2002). Zinc also has a protective effect against β-cell destruction and has anti-viral effects. Diabetics typically excrete excessive amounts of zinc in the urine and therefore require supplementation (Mooradian and Morley, 1987). Deficiency of intracellular zinc increases beta cell vulnerability to free radical attack. Restoring zinc levels in people with DM would counteract the deleterious effects of oxidative stress. In view of the positive role of zinc on insulin and beta cells, the use of these plants in the treatment of DM may be attributed to considerable amounts of zinc present in them (Naga *et al.,* 2006).

Calcium is required for normal growth and development of muscles and skeleton (Rajendra *et al.,* 2007). It improves insulin sensitivity in some type 2 diabetic

populations (Rajendra *et al.,* 2007). Calcium and cyclic AMP are important in the stimulation of insulin release. The increase in the concentration of ionized cytosolic Ca ions directly mediates the effect of glucose to stimulate insulin release from rat islet of Langerhans (Siddiqui *et al.,* 2014). Any alterations in calcium flux can have adverse effects on β-cell secretory function (Siddiqui *et al.,* 2014).

Potassium supplementation yields improved insulin sensitivity, responsiveness and secretion (Rajendra *et al.,* 2007); insulin administration induces a loss of potassium; and a high potassium intake reduces the risk of heart disease, atherosclerosis, and cancer (Norbiato *et al.,* 1984; Khaw *et al.,* 1984). Potasium depletion can result in reduced glucose tolerance (Rajendra *et al.,* 2007).

Iron influences glucose metabolism and reciprocally, iron influences insulin action (Siddiqui *et al.,* 2014). Impaired glucose metabolism and diabetes mellitus are common clinical manifestations of iron overload in patients with hemochromatosis. Recently, moderately elevated iron stores below the levels commonly associated with hemochromatosis have also been implicated in the etiology of diabetes (Acton *et al.,* 2006). Iron interferes with insulin inhibition of glucose production by the liver (Niederau *et al*., 1984).

Chromium 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 (Mooradian *et al*., 1994; Baker, 1996). Chromium administration decreases fasting and postprandial glucose and decreases fatigue, excessive thirst, and frequent urination (Cheng, 1999). The regulating or potentiating role of chromium on insulin's action has been attributed to an increase in insulin binding to cells due to an increase in the number of insulin receptors (Siddiqui *et al.,* 2014).

Copper is considered as both a powerful enzyme catalyst and a dangerous reactant that generates hydroxyl radical (Rajendra *et al.,* 2007). A deficiency of copper results in glucose intolerance, decreased insulin response, and increased glucose response. It is associated with hypercholesterolemia and atherosclerosis (Cheng, 1999). Copper possesses an insulin-like activity and promotes lipogenesis. Copper is required for absorption and transport of iron and it plays a key role in haemoglobin synthesis (Rajendra *et al.,* 2007).

Toxicity studies are fundamental in evaluation of safety of extracts or drugs used in clinical medicine. Systemic toxicity from the herbal plant extracts depends on the route and site of exposure. Because the toxicity of a drug to the host cells could render it unsuitable for therapeutic purposes, the toxicity of these plant extracts at high doses of 450mg/kg, 670mg/kg and 1000mg/kg body weight for 28 days was assessed in male Swiss albino mice. Body weight, organ weight and percent organ to body weight are one of the most sensitive drug toxicity indicators, and their changes often precede morphological changes (Michael *et al.,* 2007).

This study demonstrated that the lower rate of weekly weight gain significantly ($p \leq 0.05$) decreased as the dosage increased relative to the control following the oral and intraperitoneal administration of high doses of the plant extracts for 28 days in mice. This suggests that the extracts contained the phytoconstituents that either do not promote feed intake or interfere with its metabolism as well as enhances proteolysis of skeletal muscles retarding growth (Piero *et al.,* 2015a). Body weight maintenance can be achieved through manipulation of energy expenditure, appetite suppression, satiety enhancement and fat glucose absorption blocking. Phytochemicals found in food and herbal preparations can alter body weight, a study involving Korean pine nut oil showed that its free fatty acids significantly increased the release of satiety hormones such as cholecystokinin, delayed gastric emptying and produced subsequent increased feeling of satiety and decreased appetite (Pasman *et al.,* 2008). *G. cambogia* extracts, are reported to make meals more filling and satisfyng. The extracts may also have promoted weight reduction through suppressed fatty acid synthesis, increased lipid oxidation and reduced food intake (McCarty and Majeed, 1994).

That the observed revitalization in the body organ weights and relative organ body weights of the liver, spleen and testes following the intraperitoneal and oral administration of the plant extracts might be due to inflammation to these organs as a result of poor oxygenation (hypoxia) in these tissues increasing weight. It could also be due to the fact that plant extracts promoted higher metabolic activity and facilitated protein synthesis in these organs (Tucci, 2010).

A daily administration of high doses of 450, 670 and 1000 mg of the aqueous extracts of *C. macrostachyus, A. indica, L. javanica, O. lamiifolium* and *P. americana*, per kg body weight orally and intraperitoneally for 28 days in mice caused a significant increase in RBC, Hb, PCV and MCV and a significant decrease in MCH and MCHC levels across the four treatments. The hematological parameters RBC, PCV, and Hb are associated with the total population of the red cells; MCV reflects the size of red blood cells while MCH and MCHC are used mathematically to define the concentration of haemoglobin and to suggest the restoration of oxygen carrying capacity of the blood (Ayman, 2013). These results therefore, are an indicative of secondary polycythaemia caused by the excess production of reticulocytes and macrocytic hypochromic anemia caused by production of reticulocyte that are large in size with reduced hemoglobin concentration from the bone marrow resulting in decreased blood flow and poor tissue oxygenation (tissue hypoxia).

The immature red blod cells could be as a result of the extract constituents that interferes with folate or vitamin B_{12} absorption or by complexing with and making either of the vitamins or both biologically unavailable resulting in either folate or vitamin B_{12} deficiency. Folate and vitamin B_{12} deficiency causes DNA synthesis in all mitotic tissues to fail while the preserved RNA synthesis results in restricted cell division of the progenitor cells. Unavilability of folate or vitamin B_{12} also causes premature cell death leadig to a reduced number of mature erythrocytes (Barger, 2003).

The increased erythrocytes levels across the four treatments might have been due to myeloproliferative syndrome a primary process in the bone marrow. It may also be a reaction to chronically low oxygen levels in body tissues (Uthman, 1996; Barger, 2003). It could also indicate that the plant extracts facilitates the formation or secretion of erythropoietin, which stimulates stem cells in the bone marrow to produce red blood cells (Ohlsson and Aher, 2012). The stimulatory effect of the extracts on erythrocytes production may suggests that they exert an anemic protection. This could be as a result of their ability to prevent or inhibit lipid peroxidative system by their antioxidant activity due to presence of flavonoids (Mahmoud *et al.*, 2012), maintenance of cellular integrity, attenuation of proinflammatory cytokine production such as interleukin (IL)-6 and tumor necrosis factor (TNF)-alpha and stimulation of adiponectin expression (Ayman, 2013).

Results from this study showed that the intraperitoneal and oral administration of high dosage levels of 450, 670, and 1000 mg/kg body weight of *C. macrostachyus, A. indica, L. javanica, O. lamiifolium* and *P. americana* daily for 28 days caused an accelerated production of white blood cells and its related indices across the four treatments indicating an enhanced immunity to mice (Kaushansky, 1995; Li *et al.*, 1999). This shows that the extracts might have had good stimulation of haematopoietic regulatory elements by the macrophages and stromal cells in the bone marrow which regulate the proliferation, differentiation and maturation of committed stem cells necessary for the production of white blood cells (Howard and Hamilton, 2002).

However, administration of such high doses for 28 days in mice might be toxic to animal tissues. The increased number of WBCs, lymphocytes, neutrophils, monocytes, basophils, eosinophils and platelets count may be a sign of bodys' response protective mechanisms to an inflammatory, infectious, hypoxic, atrophic and necrotic states following the cytotoxic effects of the plant extracts (Howard and Hamilton, 2002).

Results from this study also showed that oral and intraperitoneal administration at high dosage levels of 450, 670, and 1000 mg/kg body weight of *C. macrostachyus, A. indica, L. javanica, O. lamiifolium* and *P. americana* daily for 28 days caused an increase in platelets count. Platelets are responsible for coagulation (Williams and Levine, 1982). The rise in platelets count seen may suggest that the extracts have a stimulatory effect on thrombopoietin (Li *et al.,* 1999). They, therefore, can be used in management of haemophilia. The cause of increased platelet count (thrombocytosis) and MVP in mice may be associated with inflammation and presence of a blood disease such as abnormal bleeding induced by toxic phytochemical substances such as tannins in the plant extracts.

Systemic toxicity due to a biochemical or immunological alterations of the plant extracts or drugs from the herbal extracts can lead to cellular destruction that results in direct tissue damage (Piero *et al.,* 2015a). The liver and kidneys play significant roles in various metabolic processes. The liver plays an important role in xenobiotic metabolism and the kidneys are the main organs involved in drugs elimination and therefore particularly exposed to the toxic effects of exogenous compounds (Bidhe and

Ghosh, 2004). The measurable endpoint of toxicity may be a pharmacological, biochemical, or a pathological change, which shows percentage or proportional change (Timbrell, 2009).

Results from this study showed that the intraperitoneal and oral administration of the extracts reduced levels of MHC and MCHC across treatments reflecting diminished oxygenation of tissues resulting in tissue hypoxia. Tissue hypoxia therefore, causes most body organs including brain, liver, lungs, heart, kidney, testis and intestines to initially enlarge and as the cells continue swelling, they rupture resulting in a reduced organ size (Mukundi *et al.,* 2015).

During the process of tissue hypoxia, cells rapidly deplete the store of glycogen and phosphocreatine (a source of rapid ATP production) since they solely rely on glycolysis for ATP production. As a result of this, the rate of ATP production decreases disrupting the Na^{+}-K^{+} pump responsible for maintaining the osmotic potential of the cell membrane. Therefore, the transmembrane ionic concentrationsis lost resulting in osmosis and consequent swelling of the cells and its inclusions (Murugi *et al.,* 2012). The overstretched membrane therefore, becomes permeable and leaks their enclosed contents to the blood stream. This could account for increased levels of measured biochemical profiles such as alanine aminotransferase, alkaline phosphatase, blood urea nitrogen, GGT, alpha amylase, lactate dehydrogenase, aspartate aminotransferase, creatine kinase and uric acid following treatment with 450, 670, and 1000 mg/kg body weight of the plant extracts daily for 28 days in mice.

Aminotransferases (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)) measure the concentration of intracellular hepatic enzymes that have leaked into the circulation following any damage to the parenchymal hepatocyte cells (Wolf *et al.,* 1972). They serve as markers of hepatocyte injury (Harris, 2005). Eighty percent of aspartate aminotransferase is found in the mitochondria whereas alanine aminotransferase is purely cytosolic. Raised levels of AST can be taken as a first sign of cell damage that lead to the outflow of the enzyme into the serum (Mdhululi, 2003) however, ALT is considered a more sensitive marker of hepatocellular injury than aspartate aminotransferase (Al-Mamary *et al*., 2002). The liver releases ALT and an increased plasma concentration is an indicator of liver damage.

The liver, kidney and heart release AST and ALT and elevation in their plasma concentrations indicate liver and heart damage (Ogbonnia *et al.,* 2008). The oral and intraperitoneal administration of plant extracts caused a significant increase in level of AST and ALT and maintained the normal levels observed in normal mice showing that at the doses used the extracts did not have hepato - and nephro – protective effects (Ugwu *et al.,* 2013). Therefore, it is possible to suggest that these extracts at high doses administered are not safe and might not confer protection against diabetes – induced hepatocellular damage (Ugwu *et al.,* 2013). Results indicating a decline in AST/ALT ratio across treatments are more marked for chronic hepatitis, myocardial necrosis, hepatic metastases and liver congestion (Dacie and Lewis, 1991).

On the other hand, serum bilirubin, a key pigment found in the bile, is considered a true test of liver function, as it reflects the liver's ability to take up, process, and secrete bilirubin into the bile. It also indicates hemolysis as it is the by-product of breakdown of heme. The reduced levels as observed reflects presence of secondary polycethemia (Sahil, 2013).

Results of other enzyme markers for liver and kidney function indicates that there was also an increase in levels of ALP, LDH, GGT and α-AMY thus suggesting that, at 450, 670 and 1000mg/kgbw doses, the plant extracts did adversely affect the cell structures. The alkaline phosphatase (ALP) enzyme catalyzes the hydrolysis of inorganic pyrophosphate, which is an inhibitor of vascular calcification (Yuji Shimizu *et al.,* 2013). While ALP is expressed in a variety of tissues, its concentrations are notably high in bone, liver, and kidneys (Yuji Shimizu *et al.,* 2013). However, increased serum ALP levels are associated with liver disease caused by intra or extra-hepatic cholestatis and some destruction of hepatic cell membrane (Marghoob *et al.,* 2013).

Lactate dehydrogenase (LDH) is a crucial enzyme involved in energy metabolism in muscle, facilitating the production of ATP via glycolysis during oxygen deprivation by recycling NAD+ (Neil *et al.,* 2008). The hypoxic conditions due to effects of extracts stimulated the secretion of LDH as an alternate anaerobic pathway to increase ATP production hence high levels of LDH in blood (Neil *et al.,* 2008). High elevations

of LDH are also observed for megablastic anemias, shock, renal infarction, hemolytic conditions, leukemias and liver disease (Dacie and Lewis, 1991).

Gamma-glutamyl transferase (GGT) mediates intracellular intake of extracellular glutathione which is an important component of antioxidant mechanisms (Nurbanu *et al.,* 2014). Glutathione is produced during normal metabolic processes and plays an important role in the protection of cells against oxidative stress. Infact GGT has been used for years as an index of hepatic dysfunction and marker of alcohol use (Nurbanu *et al.,* 2014). The elevated serum GGT as shown in the study indicates a hepatocellular injury caused by effects of the plant extracts.

Results of blood analytes indicate that there was significant increase in creatinine, urea, blood urea nitrogen and uric acid levels in intraperitoneally and orally administered *C. macrostachyus, A. indica, L. javanica, O. lamiifolium* and *P. Americana* extracts. Creatinine is known as an effective indicator of renal function and any rise in creatinine levels is observed if there is marked damage to functional nephrons (chronic nephritis) (Lameire *et al.,* 2005). From the study, the elevated serum creatinine level signifies impaired kidney function or kidney disease as a result of the cytotoxic effects of the extracts' constituents (Ugwu *et al.,* 2013). Blood urea nitrogen (BUN), usually correlates with creatinine i.e when creatinine levels increases BUN increases and vice versa. Blood urea nitrogen is the end product of protein breakdown. BUN levels are influenced by factors not connected with renal function or urine excretion. Creatinine is a better indicator of kidney function even though BUN

and creatinine usually rise and fall together (Michael *et al.*, 2010). Taken together, it is also possible to suggest that these plant extracts at high dose than the curative dose might directly damage the structural and functional integrities of cells in blood, liver and kidney (Ugwu *et al.*, 2013).

Uric acid (UA) is the end product of purine metabolism (Dacie and Lewis, 1991). Purines are constituents of the nucleotides in nucleic acids. Most UA is synthesized in the liver, and about one-thirds is excreted by the kidneys (reflecting only 10% not reabsorbed) (Shane, 1993). About one-third of UA is excreted in the feces. Increased UA levels as indicated in this study are marked for kidney and liver atropy, increased protein breakdown and increased levels of triglycerides (Dacie and Lewis, 1991).

The orally and intraperitoneally administered plant extracts resulted in decreased levels of various lipid parameters and those of blood glucose. This indicates that the confirmed presence of tannins in all plant extracts may have contributed to reduced feed intake by decreasing palatability, enhance satiety, suppress appetite, cause ulcers, reduce digestion, and induce fat and glucose absorption blocking (malabsorption) (Ugwu *et al.*, 2013). This lowers the bioavailability of cholesterol, triglycerides, α-lipoproteins and glucose (Ugwu *et al.*, 2013). The hepatocellular injury due to cytotoxic effects of the extracts may have halted the metabolic, excretory and defense functions of the liver hence reduced levels of serum TG, TC, LDL-C, HDL-C and glucose.

The phytoconstituents that were qualitatively determined in this study include saponins, alkaloids, terpenoids, flavonoids, sterols, tannins, free and bound antraquinones. Phytochemicals such as alkaloids, tannins, terpenoids, saponins, flavonoids and sugars have been reported to be toxic. Saponins have been reported to hemolyse red blood cells and cause cell death of many tissues (Diwan *et al*., 2000; Al-Sultan *et al*., 2003). In the kidneys, saponins lead to haemorrhage in the glomeruli and focal destruction of the renal tubules (Piero *et al.,* 2015a). Toxic levels of saponins cause cardiac failure, acute hypoglycemia and hepatorenal damage leading to death (Diwan *et al*., 2000).

Alkaloids have been reported to cause liver megalocytosis, proliferation of biliary tract epithelium, liver cirrhosis and nodular hyperplasia (Zeinsteger *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 (Piero *et al.,* 2015a). Some of the toxic alkaloids found in plants include confine, solanine, methyllycaconitine, nudicauline, and geyerline (Gardner and Pfister, 2007), 2-pentylpiperidine (Radulović *et al*., 2011), and pyrrolizidine alkaloids (Wiedenfeld, 2011). Terpenoids have been reported to increase membrane permeability to divalent and monovalent ions (Zeinsteger *et al*., 2003).

Tannins reduce feed intake by decreasing palatability and by reducing feed digestion (Piero *et al.,* 2015a). Palatability is reduced because tannins are astringent. Astringency is the sensation caused by the formation of complexes between tannins

and salivary glycoproteins. Low palatability depresses feed intake. Tannins are divided into two: hydrolysable and condensed tannins. Hydrolysable tannins are converted by microbial metabolism and gastric digestion into absorbable low molecular weight metabolites such as tannic acid which are toxic (Ugwu *et al.*, 2013).

The major lesions associated with hydrolysable tannins poisoning are hemorrhagic gastroenteritis which decreases absorption of nutrients, necrosis of the liver, and kidney damage with proximal tubular necrosis (Kumari and Jain, 2012). Protanthocyanidins (PAs) (condensed tannins) retard growth by inhibiting feed intake and digestibility (Click and Joslyn, 1969). 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 and 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 (Ugwu *et al.*, 2013).

Mineral elements are very essential for normal body functioning but are only needed in small quantities. Overdose may upset homeostatic balance or cause toxicity. Among the measured minerals were Mg, Ca, Pb, Cu, Ti, V, Cr, Br and Rb whose levels were above the recommended daily allowance. Moreover, as the administered dose increased for each extracts, the serum mineral concentration levels also went high implying increased toxicity.

In humans, Mg is required in the plasma and extracellular fluid, where it helps maintain osmotic equilibrium. It is required in many enzyme-catalysed reactions; however, excess Mg is associated with depression of the central nervous system (Mukundi *et al.*, 2015).

Overdose of calcium causes kidney stone formation (nephrolithiasis); the syndrome of hyperkalemia and renal insufficiency with or without alkalosis (referred to as milk alkali syndrome associated with peptic ulcer treatments). Too much copper is often associated with depression, hypothyroidism, anxiety and panic attacks, violent outbursts, fatigue and exhaustion. It also interferes with the body's ability to absorb iron and zinc and cause constipation. Cd and Pb are well known as toxic if their intake through ingestion or inhalation is excessive (Strain and Cashman, 2009).

Excess calcium also causes reduction in the absorption of iron, zinc, magnesium and phosphorus and therefore increasing their requirements. Calcium inhibits the absorption of iron in a dose-dependent and dose-saturable fashion (Strain and Cashman, 2009).

Manganese overdose causes manganic madness which is manifested by psychosis, hallucinations and extrapyramidal damage with features of Parkinsonism. Excess manganese inhibits dietary iron absorption because of competition for similar binding and absorption sites between nonheme iron and manganese (Strain and Cashman, 2009).

Zn, Cl, Fe, and K levels were found to be less than the recommended daily allowance implying increased free-radical activity and the increased oxidation of lipids, damaging the heart, arteries, and other integral parts of the vascular system due to Zn deficiency (Kumar *et al.,* 2005). Decreased levels of iron may have contributed to the development of anemia. Anemia is associated with blood levels of erythropoietin that are inappropriately low for the level of Hb resulting in hypoxia which represents an important risk factor for morbidity and mortality in many patients especially those with diabetes mellitus (Piero *et al.,* 2015a).

The health benefits derived from these trace element is a clear indication that their deficiencies appear to be an additional risk factor in the development and progress of disease and they contribute to the pathogenesis of diabetes mellitus and its complications. Their repletion may be an effective therapeutic intervention in prevention of the progression of the diabetes and its complications, along with a glycaemic control and control of other risk factors.

5.2 CONCLUSIONS

From this study it can be concluded that;

i. All the five plants species studied showed the antidiabetic activity when therapeutic doses were administered both intraperitoneally and orally.

ii. A comparison of the difference in antidiabetic effect of the five aqueous plant extracts in mice models at various dosages indicated that *A. indica* and *O. lamiifolium* demonstrated the highest antidiabetic effect with an optimal dose

occurring between the third and the seventh hour where the blood sugar reduction rate was between 45% and 75%.

iii. Qualitative screening of aqueous extracts of the five studied plants indicated the presence of alkaloids, flavonoids, tannins, sterols, saponins, and free and bound antraquinones which have been indicated to confer hypoglycemic effect.

iv. Elemental analysis of the five aqueous plant extracts revealed the presence of Mg, K, Ca, Mn, Fe, Zn, Br, Rb, Cr, Ti, Cu, V, Cl and Pb minerals.

v. The intraperitoneal or oral administration of high doses of each of the plant extracts (at 450, 670 and 1000mg/kg body weight) demonstrated toxic effects as evidenced by hematological and biochemical parameters changes, weekly change in body weights, changes in organ weights and relative organ to body weights of mice. Consequently, an increament in the plant extract dosage level administered was proportional to their respective toxicological effects in mice.

vi. The observed toxicological effects could account for the presence of the phytoconstituents such as tannins, alkaloids saponins, terpenoids and anthraquinones together with presence of various mineral elements at toxic levels like Mg, Pb, Ca, Cu, Ti, Cr, V, Br, Rb or at a reduced levels below RDA especially Fe, Zn, K and Cl which have demonstrated to result in toxic effects in mice.

vii. Therefore, the observed antidiabetic and toxicological effects provided the basis for the rejection of the null hypotheses.

5.3 RECOMMENDATIONS AND SUGGESTIONS FOR FURTHER STUDIES

5.3.1 Recommendations

i. The optimal dose for bioscreening antidiabetic activities was 48.4mg/kg body weight which demonstrated a blood glucose reduction rate between 66% and 71%.

ii. Administration of either*A. indica* or *O. lamiifolium* is highly recommended in the management of diabetes due to the observed high percent reduction potential in blood glucose levels for each extract in all therapeutic doses administered in both routes.

iii. There seems to be toxicological effects due to administration of extracts above the therapeautic doses hence should be avoided.

5.3.2 Suggestions for further studies

i. To undertake further research on these plants to establish the mechanism of their hypoglycemic activity to explore possibilities of developing a drug that can function by similar mode of action as the plant extracts.

ii. To investigate the antidiabetic and toxic effect of the combined dosages of these plant extracts in order to create a rationale for a combination therapy in the management of diabetes mellitus since it’s the mode of administration traditional practitioners’ use in their clinical practice.

iii. To carry out prospective studies on carcinogenic test, toxicokinetic profiling and comprehensive toxicity studies to establish the safety profile of all these plant extracts.

iv. The organic solvent extraction for these plants should be done to compare the antidiabetic activities of both aqueous and organic fractions.

v. An investigation on the effect of the herbal extracts on higher animals like apes, chimpanzees or monkeys which are physiologically closer to human beings should be done in order to find a better indication of their effects in humans.

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APPENDICES

Appendix 1: Diagnostic criteria for DM and two high risk categories of pre-DM (IFG and IGT)

	FPG(mg/dl)	**2-HPG(mg/dl)**	**Sx of diabetes +CPG**
Normal	<100	<140	-
IFG	≥100 & ≤126	-	-
IGT	-	≥140 & >200	-
Diabetes	≥126	≥200	+CPG ≥200mg

FPG- Fasting Plasma Glucose

2-HPG- 2 hour post 75g glucose load plasma glucose

CPG- Casual plasma glucose

Sx- Symptoms of diabetes e.g polydipsia, polyurea weight loss (Llorente and Malphurs, 2007)

Appendix 2: Preparation of the extracts

For efficacy studies: 25 mg/kg body weight for albino swiss mice of average weight of 23g, required, 12 mg dissolved in 2 ml of physiological saline. 48.4 mg/kg body weight required 22mg dissolved in 2ml of physiological saline. 93.5 mg/kg body weight required, 43 mg dissolved in 2 ml of physiological saline. 180.9 mg/kg body weight required, 84 mg dissolved in 2 ml of physiological saline. 350 mg/kg body weight required 162 mg of extract dissolved in 2 ml physiological saline.

There were eight groups of animals in the study as follows;

Normal controls to monitor the circadian rhythm (5 animals).

Diabetic control not treated with either oral or intravenous conventional drug or extracts (5 animals).

Diabetic group treated with either insulin or glibenclamide (5 animals).

Diabetic groups treated with:

25mg/kg body weight (5 animals).

48.4mg/kg body weight (5 animals).

93.5mg/kg body weight (5 animals).

180.9mg/kg body weight (5 animals).

350mg/kg body weight (5 animals)

For preliminary toxicity studies

1000 mg/kg body weight for albino swiss mice of average weight of 23g, required, 23 mg dissolved in 0.1% of physiological saline. There were 5 animals per group for each plant extract each requiring 115mg dissolved in 0.1% physiological saline per day.

The extract was administered for 28 days which therefore required 3220mg of the extract dissolved in 0.1% physiological saline. 670 mg/kg body weight required, 15.41 mg of the plant extracts dissolved in 0.1% of physiological saline while 450 mg/kg body weight required, 10.35 mg of the plant extracts dissolved in 0.1% of physiological saline.

Appendix 2.1: Experimental design for toxicity studies

Groups (5 mice per group)	**Treatment**	**Duration**
1	Oral control (0.1ml physiological saline)	28 days
2	Intraperitoneal control (0.1ml physiological saline)	28 days
3	Orally-Extracts each at 450,670 and 1000mg/kg body weight	28 days
4	Intraperitoneally- Extracts each at 450,670 and 1000mg/kg body weight	28 days

Appendix 3: Normal Hematology Reference Values (CDC, 2008)

PCV (%)	35.1-45.4
Hb (g/dL)	11.0-15.1
RBC (x 10^6/µL)	6.36-9.42
MCH (pg)	14.1-19.3
MCHC (g/dL)	30.2 – 34.2
MCV (fL)	45.4 – 60.3
WBC (x 10^3/L)	1.8-10.7
Neutrophil (%)	6.6-38.9
Lymphocytes (%)	55.8-91.6
Monocytes (%)	0.0-7.5

Appendix 4: Instrumental conditions for AAS

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

Parameter	**Chromium**	**Magnesium**	**Vanadium**
Wavelength (nm)	357.9	285.2	318.4
Slit width (nm)	0.7	0.7	0.2
Lamp current (mA)	5.0	5.0	5.0
Flow rate (1/min)			
Air	7.0	7.0	7.0
Acetylene	1.8	1.8	1.8

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

Appendix 5: Species voucher information

Species (Botanical identity and local Kisii name)	**Collection location**	**Collection date**	**Voucher number**
C.macrostachyus (Omososcho)	Kijauri	July 2013	WAO1
A.indica (Omwarobaini)	Kijauri	July 2013	WAO2
L, javanica (Omonyinkwa)	Kijauri	July 2013	WAO3
O. lamiifolium (Ribuko)	Kijauri	July 2013	WAO4
P. americana (Eabukato)	Kijauri	July 2013	WAO5

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Research Article Open Access

Hypoglycemic Effect of *Lippia javanica* in Alloxan Induced Diabetic Mice

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Abstract

Lippia javanica is widely distributed throughout Kenya where it is used extensively in traditional herbal preparations. An infusion of the leaves is commonly used as a decongestant for colds and coughs including diabetes, however, its efficacy profiles have not been scientifically evaluated. The aim of this study was to determine the *in vivo* antidiabetic activity of aqueous leaf extracts of this plant in white male alloxan-induced albino mice. The antidiabetic activity of the aqueous leaf extracts was orally and intraperitoneally bioscreened in alloxan induced diabetic mice at different doses of 25 mg/kgbwt, 48.4 mg/kgbwt, 93.5 mg/kgbwt, 180.9 mg/kgbwt and 350 mg/kgbwt. The treatment effects were then compared with the controls. Phytochemical composition was assessed using standard procedures. The extract showed hypoglycemic activity at dose levels of 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight in a dose independent manner. The extracts contained tannins, flavonoids, saponins, sterols, alkaloids, and free or bound anthraquinones. The observed hypoglycemic activity could be associated with the phytochemicals present in this plant extract. In conclusion the results showed that the plant extracts were effective in reducing blood sugar levels and revealed the presence of vital phytochemicals which possess antidiabetic activities. The study therefore, confirmed the traditional use of these herbs and established their efficacy data that can guide proper use of these plants in the management of diabetes mellitus. Consideration should be made to carry out the same studies using higher animals or subject the plant to organic solvent extraction and compare activities of both aqueous and organic fractions.

Keywords: Diabetes Mellitus; *Lippia javanica;* Hypoglycemic activity; *in vivo;* Antidiabetic; Phytochemicals; Insulin Units (IU)

Introduction

Diabetes is a group of metabolic disorders characterized by a chronic hyperglycemic condition resulting from defects in insulin secretion, insulin action or both [1]. The prevalence of diabetes is increasing rapidly worldwide and the [2] has predicted that by 2030 the number of adults with diabetes would have almost doubled worldwide, from 177 million in 2000 to 370 million. Experts project that the incidence of diabetes is set to soar by 64% by 2025, meaning that a staggering 53.1 million citizens will be affected by the disease [3]. The disease has several pathogenic processes ranging from autoimmune destruction of pancreatic β-cell proteins resulting in absolute insulin deficiency (Type I) to multiple abnormalities that include a combination of genetic factors related to impaired insulin secretion, insulin resistance and environmental factors such as obesity, overeating, lack of exercise and stress, as well as aging (Type II) [4].

At the onset of overt hyperglycemia the patient manifests excessive excretion of urine (polyuria), thirst (polydipsia), constant hunger (polyphagia), weight loss, vision changes and fatigue. These symptoms are more marked in type 1 diabetics [5]. Chronic hyperglycemia causes disturbances in metabolism of carbohydrates, fats and proteins resulting in long term microvascular and macrovascular complications [6]. These complications include retinopathy with potential loss of vision; nephropathy resulting in renal failure; peripheral neuropathy associated with risk of foot ulcers, amputations, and Charcot joints; and autonomic neuropathy which is recognized by gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction [6].

Normal fasting plasma glucose levels ranges between 3.5-6.7mmol/l (63-120.6 mg/dL). After a meal the blood glucose level rises to approximately 8mmoles/L and rarely exceeds this level. Repeated fasting blood glucose levels ≥ 7.0mmoles/L (126mg/dL) or 2-hour postprandial glucose values ≥ 11.1mmole/L (200mg/dL) is considered to be diagnostic criteria for diabetes and correlates with Hb A1C threshold of 6.5% [7]. .

In conventional medical practice, the current therapies of diabetes mellitus are reported to be expensive, unavailable and have side effects [8]. For example, use of insulin and oral hypoglycemic agents is associated with drawbacks such as ineffectiveness on oral administration, short shelf life, requirement of constant refrigeration and in the event of excess dosage, fatal hypoglycemia ensue. The use of oral hypoglycemic drugs like sulfonylureas and biguanides is also associated with tendency to gain weight [9]. Therefore, there is need to use effective, easily accessible and cheap means to manage diabetes mellitus.

Herbal medicines and traditional medical practitioners are receiving considerable attention from mainstream health officials, international medical research and training institutions. Traditional medicine, in the estimate of the World Health Organization is used by up to 80% of the population of most developing countries especially in Africa [10]. This is envisaged by strained economic situations of most

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Research Article Open Access

In Vivo Antidiabetic Activity of the Aqueous Leaf Extract of Croton *macrostachyus* in Alloxan Induced Diabetic Mice

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Abstract

The folklore reports from traditional medical practitioners that *Croton macrostachyus* has bioactivity against several diseases including diabetes mellitus have not been scientifically evaluated. The aim of this study was to determine the *in vivo* hypoglycemic activity of aqueous leaf extracts of this plant in male swiss white albino mice. Aqueous leaf extract of *Croton macrostachyus* was intraperitoneally and orally administered to alloxan (180.9 mg/kg; intraperitoneally)-induced diabetic mice at different doses of 25 mg/kgbwt, 48.4 mg/kgbwt, 93.5 mg/kgbwt, 180.9 mg/kgbwt and 350 mg/kgbwt and the effects on blood glucose levels investigated. The treatments effects were compared with three controls (normal, diabetic and diabetic treated with a standard antidiabetic drugs (insulin administered intraperitoneally at 1 IU/kg body weight in 0.1 ml physiological saline or glibenclamide administered orally at 3 mg/kg body weight in 0.1 ml physiological saline). Phytochemical composition of the leaf extracts were qualitatively assessed using standard procedures.The diabetic control mice showed significantly ($p<0.05$) higher fasting blood glucose when compared with normal control mice. Treatment of diabetic mice with doses of the leaf extract resulted in significantly ($p<0.05$) lower levels of fasting blood glucose. The effects of the leaf extract were comparable with the conventional drugs. However, the glucose lowering potency of this plant extract was dose independent. The aqueous leaf extracts contained tannins, flavonoids, saponins, sterols, anthraquinones and alkaloids. The observed hypoglycemic activity could be associated with the phytochemicals present in this plant extract. Therefore, the results suggest that *Croton macrostachyus* leaf extract is a potent hypoglycemic agent and this validates their folkloric usage. Further studies to investigate the mechanism of action for hypoglycemic activity for these plant species should be done in order to explore possibilities of developing a drug that can function by similar mode of action as the plant extract.

Keywords: Diabetes mellitus; *Croton macrostachyus*; Hypoglycemic activity; Antidiabetic; Phytochemicals

Introduction

Diabetes mellitus is a metabolic disorder characterized by high blood sugar levels that results from either an inherited or acquired deficiency in the production of insulin by the pancreatic islet cells of Langerhans or by the ineffectiveness of the insulin produced at the peripheral tissues [1]. Diabetes mellitus is a chronic medical condition which though can be controlled lasts a lifetime [2]. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels [3]. An estimated 171 million people were suffering from diabetes in 2000, and this number could total 366 million by 2030 [4]. In 2012, 1.5 million deaths were reported to be directly caused by diabetes [5].

Diabetes mellitus can be categorized into type I or insulin-dependent diabetes mellitus (IDDM) also referred to as juvenile onset diabetes resulting from a cellular mediated autoimmune destruction of β-cells in the pancreas [6]. It accounts for 5-10% globally of individuals with diabetes. The other category is type II or non-insulin-dependent diabetes mellitus (NIDDM) whose onset is usually after 40 years of age [6] and accounts for approximately 90-95% of the diabetes mellitus cases world-wide. It is also called adults onset diabetes which affects individuals who have insulin resistance and usually have relative insulin deficiency [6].

The clinical manifestation of diabetes mellitus include excessive excretion of urine (polyuria), thirst (polydipsia), constant hunger (polyphagia), weight loss, blurred vision and fatigue [5]. Acute, life threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or the non-ketotic hyperosmolar syndrome [7]. Long term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations, and Charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction [7].

Normal fasting plasma glucose levels ranges between 3.5-6.7 mmol/l (63-120.6 mg/dl). After a carbohydrate meal the blood glucose level rises to approximately 8 mmoles/L and rarely exceeds this level. Repeated fasting blood glucose levels ≥ 7.0 mmoles/L (126 mg/dl) or 2 hour postprandial glucose values ≥ 11.1 mmole/L (2000 mg/L) is considered to be diagnostic criteria for diabetes and correlates with Hb A1C threshold of 6.5% [8].

The mainstay of non-pharmacological treatment of diabetes is

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Hypoglycemic Effect of Ocimum Lamiifolium in Alloxan Induced Diabetic Mice

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Abstract

Plant remedies are the mainstay of treatment in underdeveloped regions owing to the side effects, unavailability and unaffordability of the conventional therapy. Among the traditional plants that have been used as an alternative therapy for diabetes mellitus is *Ocimum lamiifolium*, however, it has received limited scientific and medical evaluation to assess its efficacy. In this study, the *in vivo* hypoglycemic activity of aqueous leaf extracts of this plant was determined in male swiss white albino mice. The antidiabetic activity was screened in alloxan induced diabetic mice using oral and intraperitoneal routes. The phytochemical composition was assessed using standard procedures. The extract showed hypoglycemic activity at dose levels of 25, 48.4, 93.5, 180.9, and 350 mg/kg body weight. The extracts contained tannins, sterols, flavonoids, saponins, terpenoids, and alkaloids. The observed hypoglycemic activity could be associated with the phytochemicals present in this plant extract.

Keywords: Diabetes Mellitus; *Ocimum lamiifolium;* Hypoglycemic activity; Antidiabetic; Phytochemical; Toxicity

Introduction

Diabetes mellitus (DM) is a major public health problem with an estimated global incidence of 382 million diabetics by 2014 and this number is expected to increase to over 592 million people in less than 25 years [1]. Diabetes mellitus (DM) is characterized by chronic hyperglycaemia resulting from defects in insulin metabolism and impaired function in carbohydrate, lipid and protein metabolism that leads to long-term complications [2]. DM is no longer a disease of rich developed countries [3]. Changes in dietary habits, obesity and physical inactivity are responsible for spreading this epidemic into the developing countries [3].

Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss [4]. In its most severe forms, ketoacidosis or a non-ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death. Often symptoms are not severe, or may be absent, and consequently hyperglycemia of sufficient degree to cause pathological and functional changes may be present for a long time before the diagnosis is made [4]. Among the complications associated with diabetes mellitus include microvascular complications which mainly affect the retina, kidney and peripheral nervous system and may progress to more overt serious complications, and macrovascular complications, mainly atherosclerosis, that may lead to cerebrovascular ischemia and stroke [3,4].

Several pathogenic processes are involved in the development of diabetes. These include processes which destroy the beta cells of the pancreas with consequent insulin deficiency (Type I diabetes), and others that result in resistance to insulin action or both (Type II diabetes) [4]. The abnormalities of carbohydrate, fat and protein metabolism are due to deficient action of insulin on target tissues resulting from insensitivity or lack of insulin [4].

Pharmacological treatment of diabetes mellitus is based on oral hypoglycaemic agents and insulin injection which have so many side effects, coupled with its high cost which is not affordable in poor economic communities [5]. Consequently, in rural parts of worldwide societies, traditional remedies from plant sources with minimal side effect are frequently employed to manage the disorder [5].

Ocimum lamiifolium is a perennial evergreen shrub having oblong, ovate green colored leaves (0.5-5 m), oppositely arranged having pubescent leaf surface, narrow at the base and deeply serrated (Plate 1) [6]. The genus Ocimum is cultivated for its extraordinary essential oil which display many therapeutic usages such as in medicinal application, herbs, culinary, perfume for herbal toiletries, aromatherapy treatment and as flavoring agent [6]. It has wide range of therapeutic effects like antimicrobial, antispasmodic, bactericide, carminative, anthelmintic, hepatoprotective, antiviral, larvicidal, remedy of coughs, colds, measles, abdominal pains, diarrhea, insect repellent, particularly

Plate 1: *Ocimum lamiifolium* (photograph taken in July 2013 at Kijauri Nyamira County)

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Review Open Access

Hematological Markers of *In Vivo* Toxicity

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Abstract

In modern medical practice, toxicity studies are essential in assessment of safety of extracts or drugs used in clinical medicine. The interaction of the toxin or its metabolite with cellular constituents may bring about significant changes in hematological parameters. These alterations may be rapid or slow and often cause a change in structure and function of the affected tissues. In clinical laboratory setting these blood indices are assayed for the purpose of diagnosis, treatment or prevention of disease, and for greater understanding of the disease process. Therefore, assessment of hematological indices can be diagnostic of adverse effects of foreign compounds on the blood constituents since such hematological alterations have higher predictive value for human toxicity when the data are interpreted from animal studies.

Keywords Hematological markers; *In vivo* toxicity

Introduction

Systemic toxicity from therapeutic synthetic drugs and herbal medicines depends on the route of administration and site of exposure [1]. Cellular destruction causes direct tissue damage and this may have a biochemical, hematological or immunological basis. Many pathological lesions are of unknown mechanisms especially in the intermediate stages between the interaction of the toxin or its metabolite with cellular constituents and the start of the final degenerative changes that leads to cell death. Toxic effects may be detected using clinico-chemical analysis of body fluids or by gross pathological examination in the post mortem [2].

An increasing amount of attention is being directed towards the development and understanding of biological markers of *in vivo* toxicity. The biological markers enable the characterization of patient populations and quantitation of the extent to which therapies reach intended targets, alter proposed pathophysiological mechanisms and achieve clinical outcomes [3]. The potential to use biomarkers for identifying patients that are more likely to benefit or experience an adverse reaction in response to a given therapy, and thereby better match patients with therapies, is anticipated to have a major effect on both clinical practice and the development of new drugs and diagnostics [4]. Biomarkers can stratify patient populations or quantify drug benefit in primary prevention or disease-modification. Clinically, such biomarkers are required to inform regulatory and therapeutic decision making regarding the candidate therapies as they can directly contribute to detecting, quantifying and understanding the deleterious effect of exposure to a toxin [4].

The assessment of hematological parameters can be diagnostic of adverse effects of foreign compounds on the blood constituents of an animal [5]. Administration of the chemical compounds at toxic doses often results in changes in blood parameters that are indicative of hematological disorders such as anemia which is characterized by low hemoglobin content [6]; neutropenia which occurs in cases of reduced production of white blood cells or increased utilization and destruction, or both [7]; thrombocytopenia which precedes a reduction in the platelet count as a result of decrease in platelet production, decline in platelet survival, and dilution of platelet numbers resulting from transfusion of platelet-poor blood; and malignancies such as leukemia, lymphoma and myeloma [8,9].

Blood, a carrier of metabolic products from and to the various regions of the cardiovascular system, is affected by the clinical status of the tissue environment [10]. Due to the addition of altered biochemical and tissue products in the blood and their interactions with the blood constituents, the functional properties of hematological parameters are changed [11]. Blood parameters are key indicators in diagnosing the actual physiological status of an organism [12]. An organism must keep its blood composition and constituents relatively constant under natural conditions to function properly [12]. This article reviews the hematological parameters that are diagnostic of blood disorders and their alterations due to deleterious effect of the toxin since hematological changes have higher predictive value for human toxicity [13].

Erythrocytes and Related Parameters

Red blood corpuscles (RBCs)

Red blood corpuscles (erythrocytes) are enucleate cells that are packed with the oxygen-carrying protein, hemoglobin. Under normal conditions, the concentration of erythrocytes in blood is approximately 3.85-5.16 million/μL in women and 4.54-5.78 million/μL in men [14]. A decrease in number of red cells in the blood is often associated with development of anemia [14]. This could be due to the stimulation of lipid peroxidative system by the toxin resulting in production of lipid peroxides which hemolyses the RBC's especially in diabetic patients [15]. The major pathological consequences of free radical induced membrane lipid peroxidation include increased membrane rigidity, attenuation/inhibition of anti-inflammatory cytokine production like the adiponectin, increased cellular deformation, reduced erythrocyte survival via increased self-necrosis, and increased lipid fluidity which

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Research Article Open Access

Mineral Elements Content of Selected Kenyan Antidiabetic Medicinal Plants

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Abstract

The use of herbal medicine as an unconventional health treatment is gaining considerable recognition and popularity worldwide. Despite skepticism and a lack of medical evidence to support its therapeutic efficacy, use of herbal remedies has considerably increased. Belief in the superiority of herbs is based mainly on anecdotal evidence, paraherbalism, and pseudoscience. It is only recently that guidelines for their investigation have been developed and a few herbs have been clinically studied. Many diseases including diabetes mellitus has experimentally been shown to be managed by medicinal plant extracts. The hypoglycemic potential of such plants maybe attributable to the mineral elements present in them.

This study was designed to determine the content of mineral elements in five Kenyan antidiabetic medicinal plants traditionally used to manage diabetes mellitus using Total Reflection X-ray Fluorescence (TXRF) System and Atomic Absorption Spectroscopy (AAS) techniques. The elements Mg, K, Ca, Mn, Fe, Zn, Br, Rb, Cr, Ti, Cu, V, Cl and Pb were identified and their contents estimated. The results of the present study provide justification for the usage of these medicinal plants in the management of diabetes mellitus.

The results indicates that the analyzed medicinal plants can be considered as potential sources for providing a reasonable amount of the required elements other than diet to diabetic patients. Moreover, these results can be used to set new standards for prescribing the dosage of the herbal drugs prepared from these plant materials.

Keywords: TXRF; AAS; Diabetes mellitus; Trace elements; Mineral elements

Introduction

Diabetes mellitus describes a group of metabolic disorders characterized by high blood sugar levels and glucose intolerance resulting from defects in insulin secretion, insulin action, or both [1]. The chronic hyperglycemic condition is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels [1]. Several pathophysiological processes are involved in the development of diabetes mellitus [2]. These range from autoimmune destruction of the β-cells of the pancreas [3] with consequent insulin deficiency to abnormalities that result in resistance to insulin action [3]. Deficiency and insufficient action of insulin on target tissues leads to carbohydrates, fats and proteins metabolism abnormalities [4].

The presenting symptoms of hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision [5]. In its most severe forms, ketoacidosis or a non-ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death [5]. A long standing metabolic derangement is frequently associated with permanent and irreversible functional and structural changes in the cells of the body, with those of the vascular system being particularly susceptible. For instance, retinopathy results in potential loss of vision; nephropathy leads to renal failure; peripheral neuropathy increases risk of foot ulcers, amputations, and Charcot joints; and autonomic neuropathy causes gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction [6].

The rapidly increasing diabetes mellitus is becoming a serious threat to mankind health in all parts of the world. The mainstay of non-pharmacological treatment of diabetes is diet and physical activity [7]. However, in some cases control and treatment of diabetes and its complications requires conventional therapeutic applications of both insulin and oral hypoglycemic drugs [8]. Conventional management of diabetes is expensive and therefore not affordable by many patients especially in developing nations. More so, conventional drugs are not readily available and have been found to have side effects with long term of use [9]. Control and treatment of diabetes and its complications mainly depend on the chemical or biochemical agents, but the fact is that it has never been reported that someone had recovered totally from diabetes. However, the distinctive traditional medical opinions and natural medicines have shown a bright future in the therapy of diabetes mellitus and its complications.

The hypoglycemic potential of herbal remedies maybe attributable to the mineral elements present in them [10]. These micronutrients are very essential and required by the body in trace amounts or tiny quantities on a day to- day basis in order to function properly. Micronutrients can be categorized into, among others, the macro elements and trace elements. The macro elements include chloride, calcium, phosphorous, magnesium, sodium, potassium, and iron. The trace elements include cobalt, boron, chromium, copper, sulfur, iodine, fluoride, selenium, manganese, zinc, and molybdenum [11].

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Research Article Open Access

In Vivo Safety of Aqueous Leaf Extract of *Lippia javanica* in Mice Models

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Abstract

Rural dwellers in Kenya often resort to herbal remedy and dietary control in the treatment of several diseases including diabetes mellitus (DM), hypertension, cancer and cardiac diseases. The therapeutic applications of such plants has largely rested upon their long-term clinical experience, however, their safety profiles has not been well evaluated. The present study aimed at determining the *in vivo* toxic effects of orally and intraperitoneally administering *Lippia javanica* leaf extract at dosage levels of 450 mg/kgbwt, 670 mg/kgbwt and 1000 mg/kgbwt daily for 28 days on the body and organ weights, hematological indices and biochemical parameters in normal male swiss white albino mice. During this period, the mice were allowed free access to mice pellets and water *ad libitum* and observed for signs of general illness, change in behavior and mortality. Phytochemical composition was assessed using standard procedures. The oral and intraperitoneal administration of 450 mg/kgbwt, 670 mg/kgbwt and1000 mg/kg body weight of the extract decreased the body weight gain and altered the organ to body weight percentage of the brain, kidney, liver, heart, testes and lungs. Oral and intraperitoneal administration of the same doses caused a change in levels of RBC, WBC, Hb, PCV, PLT, MPV, MCV, MCH, MCHC, neutrophils, lymphocytes, eosinophils, basophils, monocytes and biochemical parameters: AST, ALP, ALT, GGT, CK, α-AMYL, LDH, T-BIL, D-BIL, I-BIL, TG, TC, LDL-C, HDL-C, BUN, UA, Urea and Creatinine. The extracts contained alkaloids, sterols, terpenoids, flavonoids, tannins and saponins.

Keywords: Diabetes mellitus; Hypertension; Cancer; Cardiac diseases; *Lippia javanica*; Toxic; *In vivo*; Mg/kgbwt; *Ad libitum*

Introduction

Toxicity studies are fundamental in evaluation of safety of extracts or drugs used in clinical medicine [1]. The short or long-term administration of a chemical compound may bring about significant changes in the function, metabolic transformation, structure and concentration of biomolecules, enzymes and even metabolic pathways [2]. These alterations might be rapid or slow and may lead to different biochemical mechanism of the drug producing similar pathological, clinical and laboratory findings [3]. Therefore, the measurable endpoint of toxicity may be a pharmacological, biochemical, or a pathological change, which shows percentage or proportional change [4].

The current pharmacological treatment of various diseases is based on oral and injectable agents which have so many side effects, coupled with their high costs which are not affordable in poor economic communities [5]. Consequently, in rural parts of worldwide societies, traditional remedies from plant sources with minimal side effect are frequently employed to manage such diseases and disorders [5].

Lippia javanica (the lemon bush) (Figure 1) is an erect, multi-branched, woody shrub that grows 1-2 m tall. The 3-4 cm long leaves are hairy on sides and have dentate, lightly toothed, margins, and are rough to the feel with deeply sunken veins from above [6]. Leaves are opposite, often in whorls of up to four and have a characteristic lemon scent when crushed. Small creamy white flowers clustered together in dense, round spikes about 1 cm in diameter are produced between February and May (but can be found throughout the year). Seeds are small brown nutlets [6].

The plant has been exploited since pre-historic time by traditional herbalists for the treatment of various ailments including diabetes, coughs, asthma, colds, 'flu, chest complaints, scabies and scalp infections and malaria [6]. Clinical studies using human volunteers have also shown that Lemon Bush extract is a more potent malaria vector mosquito repellant than most available commercial formulation

Figure 1: *Lippia javanica* (photograph taken in July 2013, at Kijauri Nyamira County).

[6]. Shikanga et al. [7] reported the presence of substantial levels of alkaloids, flavonoids, terpenes, saponins, phenolics, anthraquiniones, phlobatannins, cardiac glycosides and tannins in the leaf. One unique characteristic of the leaf is that it has bitter lemon taste. For instance, some leafy vegetables with bitter taste have been implicated in enhancing insulin production in experimental diabetic rats and have potentials for diabetic control and management [8].

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Biochemical Markers of *In Vivo* Hepatotoxicity

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Abstract

A chemical compound, whether of natural or of synthetic origin, brings about a toxicological effect when its dose is high enough or the duration of exposure is sufficient to cause an alteration in the normal homeostasis of body fluids and tissues. Therefore, the right dose differentiates a toxicant from a remedy. The body detoxifies drugs and other chemical compounds through key organs such as the liver. The liver plays a central role in the metabolism and excretion of xenobiotics which makes it highly susceptible to their adverse and toxic effects. These effects can be manifested in the form of hepatic injuries, which take many forms from cellular degeneration and necrosis to cirrhosis or cholestasis to vascular injury. Exposure to hepatotoxicants alters the homeostatic balance of various biological markers that provides a powerful and dynamic approach to understanding the spectrum of liver diseases. These markers offer a means for homogeneous classification of a disease and risk factor, and they can extend one's basic information about the underlying pathogenesis of disease and in drug design.

Key words:

Biological markers; *In vivo* toxicity; ALT; AST; ALP; γ GT; LDH; CK; Bilirubin; α-Amylase; Cholesterol and glucose

Introduction

The liver is the largest organ of the human body that performs multiplicity of vital metabolic functions [1]. Anatomically, the liver is located slightly beneath the diaphragm and anterior to the stomach [1]. It is involved in maintenance of glucose homeostasis, secretion of lipoproteins, and excretion of bile. It synthesizes albumin, prothrombin, fibrinogen and binding protein for iron, copper and vitamin A. Liver parenchyma serves as a storage organ for glycogen, fat and fat soluble vitamins, iron and copper. Catabolically, it is involved in breakdown of various hormones, serum protein, and detoxification of drugs, chemicals and products of bacterial metabolism [2]. The portal vein nourishes the liver with blood containing digested nutrients from the gastrointestinal tract, spleen and pancreas, while the hepatic artery supplies oxygenated blood from the lungs [3]. Approximately 10% to 15% of the total body blood supply will be found in the liver at any one time making it the site most vulnerable to chemical induced toxic injury either through acute or chronic exposures. Ingested metals, drugs and environmental toxicants are the major sources of hepatic injury [3]. Hepatotoxicity may result not only from direct toxicity of the primary compound but also from a reactive metabolite or from an immunologically-mediated response affecting hepatocytes, biliary epithelial cells and/or liver vasculature [4].

Exposure to hepatotoxicants alters the homeostatic balance of various biological markers in body fluids and tissues. Besides, drug induced hepatic injuries have become a major challenge for the pharmaceutical industry and public health, since those injuries are a common cause of drug development termination, drug restrictions, and post-marketing drug withdrawals [4]. Such chemical compounds at some dosages exhibit various toxicological effects or alter the toxicity of concomitantly administered therapeutic drugs [5]. Therefore, clinical evaluation of various biological markers provides a powerful and dynamic approach to understanding the spectrum of diseases. These markers, also, offer a means for homogeneous classification of a disease and risk factor, and they can extend one's basic information about the underlying pathogenesis of disease and in drug design [6].

An ideal marker of xenobioticinduced hepatic toxicity must have a substantial tissue to plasma ratio; is of liver origin (exclusively or predominantly), or its level is affected by a change in liver function (tissue specific); can be reliably measured at sublethal doses of a xenobiotic (highly sensitive); should persist in plasma for several hours to provide a convenient diagnostic time window but not so long that recurrent injury would not be identified (reliability); and it must be easily confirmed to be associated with histopathological or functional changes in the liver (relevancy) [7]. The factors that determine these characteristics and the sensitivity and specificity of each marker are size, cellular localization, solubility, release ratio, clearance, specificity for irreversible injury, and detectability [8].

In this paper, we review various biochemical parameters measured in body fluids that can directly contribute to detecting, quantifying, and understanding the significance of exposure to hepatotoxicants [9,10]. However, the final interpretation of the results of clinical investigations, whether biochemical or of any other category, should be in total context of the disease process and the clinical profile of the patient.

Liver Toxicity

Zimmerman identified four major categories of serum enzymes based on their specificity for and sensitivity to different types of liver injury [11]. The first group contains enzymes such as alkaline phosphatase (AP), 5'-nucleotidase (5'-NT), and gamma glutamyltranspeptidase (GGT) whose elevation in serum appear to reflect cholestatic injury [11]. In contrast, the second group of enzymes includes those that are more sensitive to cytotoxic hepatic injury and are further subdivided into:

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Medicinally Important Phytochemicals: An Untapped Research Avenue

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Keywords: Phytochemicals; Flavonoids; Alkaloids; Sterols; Stilbenes; Lignans; Saponins.

ABSTRACT

The past decade has witnessed a tremendous resurgence in the interest and use of medicinal plants. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in them known as phytochemicals. Phytochemicals are biologically active, naturally occurring chemical compounds found in fruits, vegetables, grains, nuts, tea and seeds that promote human health and prevent diseases. The therapeutic effects of these medicinal plants can justifiably be attributed to, among others, the phytochemicals in them especially the flavonoids, alkaloids, sterols, terpenoids, phenolic acids, stilbenes, lignans, tannins and saponins. The abundance of scientific evidence indicates that such bioactive compounds have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property. This paper avails a review of medicinally important plant-derived compounds that can be used in design of more efficacious therapeutic agents against many communicable and non-communicable diseases.

INTRODUCTION

In recent years, herbal prescriptions have received considerable attention as an alternative way to compensate for perceived deficiencies in orthodox pharmacotherapy worldwide [1]. Despite a lack of medical evidence to support their therapeutic efficacy and toxicological effects, the use of herbal medicine has increased considerably [1]. According to World Health Organization (WHO), up to 80% of the world's population in underdeveloped and developing countries relies on traditional medicine practices for their primary health care needs [2]. Traditional medicines have been accorded greater acceptance in Africa because of the unavailability, unwanted side effects and high costs associated with orthodox medicines, inadequate health facilities and healthcare professionals, coupled with inadequate training of health workers [3]. The therapeutic effects of these medicinal plants can justifiably be attributed to, among others, the phytochemicals in them especially the flavonoids, alkaloids, sterols, terpenoids, phenolic acids, stilbenes, lignans, tannins and saponins.

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack [4]. These compounds are known as secondary plant metabolites and provide health benefits to humans. They are thought to act as synergistic agents, allowing nutrients to be used more efficiently by the body. Some of the beneficial roles of phytochemicals are low toxicity, low cost, easy availability and their biological properties such as antioxidant activities, antimicrobial effects, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and antineoplastic properties [5].

Phytochemicals are not essential nutrients and are not required by the human body for sustaining life, but have important properties to prevent or to fight some common diseases [6]. Because of this property; many studies have been undertaken to