ANTIDIABETIC ACTIVITY AND SAFETY OF *Maerua decumbens* METHANOLIC ROOT EXTRACT IN WISTAR RATS

BY

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN BIOCHEMISTRY IN THE SCHOOL OF SCIENCE

UNIVERSITY OF ELDORET, ELDORET, KENYA

OCTOBER, 2018

DECLARATION

Declaration by the Candidate

This thesis is my original work and has not been submitted for any academic award in any institution; and shall not be reproduced in part or full, or in any format without prior written permission from the author and/or University of Eldoret.

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Declaration by Supervisors

This thesis has been submitted for examination with our approval as University supervisors.

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DEDICATION

This thesis is dedicated to my dear wife Sheilla and to my loving sons; Titus, Elias and Ryan, for their daily commitment and unending inspiration throughout this great exercise. God bless you.

ABSTRACT

Diabetes mellitus (DM) is increasingly affecting many people worldwide. Use of conventional antidiabetic drugs is expensive and is also associated with undesirable side effects that necessitates the need to find alternative drugs which are affordable and with minimal side effects. Despite the wide use of herbal medicines as an alternative especially in developing countries, there is inadequate scientific evidence on their antidiabetic activity and safety. For instance, there is no scientific evidence on the antidiabetic claims of *Maerua decumbens* roots and also its safety. Therefore, this study aimed at evaluating oral toxicity and antidiabetic activity of *M. decumbens* methanolic root extract in Wistar albino rats. Qualitative phytochemical analysis of the extract was also assessed. In acute toxicity study, extract (2000 mg/kg b.wt) was orally administered once to female rats and monitored for 14 days. In sub-acute toxicity study, the extract was administered daily at 400 and 800 mg/kg to rats of both sexes for 28 days. Weekly b.wt were determined and at the end of the treatment period, rats were sacrificed, organ weights (liver, kidney, heart and spleen) recorded, serum indices of liver and kidney function, hematological parameters, liver and kidney histology were performed. In antidiabetic study, streptozotocin (50 mg/kg) induced diabetic rats were treated with *M. decumbens* extract (100 and 400 mg/kg) and metformin (100 mg/kg) for 21 days and fasting b.wt and blood glucose (FBG), liver malondialdehyde (MDA) levels, and histology of liver and pancreas were investigated. Statistical analyses were done using Student t-test and Analysis of Variance ($p<0.05$). Phytochemical screening showed presence of alkaloids, glycosides, flavonoids, saponins, steroids, tannins and terpenoids. In toxicity studies, extract-treated rats showed no signs of toxicity or mortality and b.wt versus the normal control. The relative organ weights showed marginal differences while serum indices of liver and kidney function showed normal levels except significant decreases in total protein and urea levels in extract-treated rats versus normal control. There was no notable change in the White Blood Cells' differential count, while Red Blood Cells' indices showed significant alterations but their values remained within published normal reference ranges for the species. In antidiabetic study, extract-treated diabetic rats showed a marginal increase in b.wt, significant decreases in FBG and MDA, and cytoprotection of liver and pancreas versus untreated-diabetic rats. Therefore, *M. decumbens* root extract administered orally in rats is safe coupled with antidiabetic activity that provides great potential for its use as medicine for DM.

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LIST OF ABBREVIATIONS

ADA	American Diabetes Association
ANOVA	Analysis of Variance
B.wt	Body Weight
DM	Diabetes Mellitus
DPP	Diabetes Prevention Program
FDA	Food and Drug Administration
G	Grams
GDM	Gestational Diabetes Mellitus
HELB	Higher Education Loans Board-Kenya
IDDM	Insulin Dependent Diabetes Mellitus
IDF	International Diabetes Federation
MD	*Maerua decumbens*
MDA	Malondialdehyde
Mg/kg	milligrams per kilogram
NIDDM	Non Insulin Dependent Diabetes Mellitus
NRF	National Research Fund-Kenya
OECD	Organization for Economic Development and Cooperation
RPM	Revolutions per Minute
STZ	Streptozotocin
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
TBA	ThiobarbituricAcid
WHO	World Health Organization

ACKNOWLEDGEMENT

First and foremost, I would like to express my sincere gratitude to my supervisors Dr. Vivian Tuei and Dr. Geoffrey Maiyoh for their keen interest, continuous support during my research, for their motivation, enthusiasm and invaluable advice in supervising my thesis work. I would also like to thank Dr. Benson Macharia of Moi University for his professional support in histological analyses and interpretation of the results. I am truly grateful.

I would like to thank National Research Fund-Kenya (NRF) for funding my research project (2016/2017 MS Awards). I would like to also thank Higher Education Loans Board-Kenya (HELB) for their scholarship in sponsoring my postgraduate tuition. I wish to extend my thanks to the Board of Postgraduate Studies, Directorate of Research and Innovation, School of Science and Department of Chemistry and Biochemistry of the University of Eldoret for their academic support.

I wish to also thank my colleagues at the University of Eldoret for their encouragement throughout this academic journey. Last but not least, I am forever indebted to my parents, brothers and sisters for supporting my academic pursuits this far both morally and financially.

Above all, glory to almighty God for giving me the strength, good health and sound mind to accomplish this study.

CHAPTER ONE

INTRODUCTION

1.1 Background

The term diabetes mellitus (DM) describes a metabolic disorder of multiple etiologies characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long term damage, dysfunction and failure of various organs. Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss (WHO, 2013). In its most severe forms, ketoacidosis or a non–ketotic hyperosmolar state may develop and lead to stupor, coma and even death in absence of effective treatment (WHO, 1999).

The long term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation, Charcot joints, and features of autonomic dysfunction, including sexual dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease (IDF, 2013). Diabetes can be divided into two main groups based on their requirements of insulin; type 1, insulin dependent diabetes mellitus (IDDM); type 2, non-insulin dependent diabetes mellitus (NIDDM); others include, type 3, maturity onset diabetes of the young (MODY); type 4, gestational diabetes. Type 2 diabetes accounts for 90 percent of diabetic cases and is characterised by insulin resistance and β-cell dysfunction (ADA, 2010).

The prevalence of diabetes was estimated at 382 million people in 2013 and it is projected to rise to 592 million by the year 2035 (IDF, 2013). With this high prevalence, the highest mortality due to diabetes mellitus occurs in low and middle income countries, where four out of every five persons with diabetes globally live in developing countries and the most affected are men and women of working age. The current rapid global rise in diabetes rate is attributed to the rapid rise in sedentary life styles, urbanization, nutrition transitions and aging (Hu, 2011).

The WHO has predicted that the major burden of diabetes will occur in developing countries. Life expectancy may be halved by diabetes mellitus especially in developing countries where its prevalence is increasing and adequate treatment is often unavailable due astronomical costs (Dhasarathan & Theriappan, 2011). The only available pharmacological intervention for Type 1 diabetes is insulin (Gough & Narendran, 2010). Conversely, therapeutic strategies for type 2 diabetes involve insulin and four main classes of oral antidiabetic agents that stimulate pancreatic insulin secretion (sulphonylureas and rapid-acting secretagogues/insulinotropics like glibenclamide, glipizide, rapaglinide), reduce hepatic glucose production (biguanides like metformin), delay digestion and absorption of intestinal carbohydrate (α-glucosidase inhibitors like acarbose) or improve insulin action (thiazolidinediones like pioglitazone, rosiglitazone) (Kimani *et al*., 2015). Each of above anti-diabetic agents suffers from generally inadequate efficacy and number of serious adverse effects besides also them being costly (Bailey, 2008). The streptozotocin (STZ)-induced Wistar rat model for type 1 diabetes is a good model that has been used extensively in animal models to study both the

pathology of diabetes mellitus and complications related to the disease as well as possible interventions (Furman, 2015).

With regard to the above limitations of conventional drugs, there is need to explore other treatment and management strategies of diabetes mellitus (Pareek *et al*., 2009). Therefore, the search for more effective and safer hypoglycaemic agents is one of the important areas of research, and mankind has long been using herbs for the treatment of various ailments since they are less costly, show better tolerance, safer, with less side effects, are widely available, and are ecofriendly in nature (Choudhary & Pawar, 2014). Management of diabetes using traditional remedies is widespread in Africa in rural as well as urban communities. An increasing number of patients who opt for traditional remedies are driven by a combination of factors for instance financial constraints, geographical accessibility to the population, inadequate healthcare systems, ease of accessibility of traditional medicine, indigenous knowledge of community members as well as the role of traditional healers (WHO, 2013).

According to World Health Organization (WHO), up to 80% of the world's population in developing countries relies on traditional medicine practices for their primary health care needs, with over 90% of the Kenyan population relying on herbal medicines (Abdirahman *et al*., 2015). Plants contain a great diversity of bioactive compounds which makes them a possible source for different drugs (Mahmood *et al*., 2012). Many herbs and plants have been described as possessing hypoglycaemic activity when taken orally. According to WHO, there are more than 1200 plant species worldwide which are used in the treatment of diabetes mellitus (Olaniyan *et al.*, 2015). Most plants contain phytochemicals such as glycosides, alkaloids, terpenoids, flavonoids, and carotenoids

which have been implicated to contain antidiabetic activity (Malviya *et al*, 2010). The drug Metformin which is widely used as a hypoglycemic agent was originally derived from the medicinal plant *Galega officinalis* (Piero *et al.*, 2012). Some of these herbs have been proven to provide symptomatic relief and assist in the prevention of the secondary complication of diabetes, while others helped in the regeneration of β-cells and in overcoming insulin resistance (Pandey *et al.*, 2011).

Maerua decumbens (Brongn) De Wolf is an evergreen shrub growing in arid and semi-arid habitats. Local communities living in rural regions of Kenya use the roots to purify water, the fruits are edible and the leaves used to heal sore joints (Beentje *et al.*, 1994). The Keiyo communities in Kenya traditionally claim to use the boiled roots or freshly chewed roots to treat diabetes (Kigen *et al.*, 2014). The claim on this antidiabetic activity or safety of *M. decumbens* roots form the basis of this study since it has not been tested, proven or documented scientifically. Therefore, this study aimed to investigate the antidiabetic activity of M*aerua decumbens* methanolic root extract in STZ-induced diabetic rats and and its safety in normal rats.

1.2 Statement of the Problem

According to a recent estimation by the WHO, diabetes mellitus has a global prevalence of 9% among adults. The disease caused over 1.5 million deaths in 2012 and WHO projects that diabetes would be the 7th leading cause of death by the year 2030 (Whiting *et al.*, 2011). The WHO estimates that the prevalence of diabetes in Kenya is at 3.3% (Chege, 2010), and it is predicted to rise to about 4.5% by the year 2025 (Mcferran, 2008). What is even more disturbing is that two-thirds of diabetics may be undiagnosed (Beran & Yudkin, 2006) and the disease may greatly hamper Kenya's progress towards

achieving her sustainable development goals in health care (Wamai, 2009). Although diabetes is sometimes considered a condition of developed nations, the loss of life from premature death among persons with diabetes is greatest in developing countries. In these countries three-quarters of all people with diabetes are under 65 years old and 25% of all adults with diabetes are younger than 44 years (WHO, 2016). Having diabetes is associated with substantially higher lifetime medical expenditures despite being associated with reduced life expectancy. If prevention costs can be kept sufficiently low, diabetes prevention may lead to a reduction in long term medical costs. A recent study estimates that a diabetic patient on average spends about KES 349, 800 annually (Hall *et al.*, 2011).

The management of diabetes is a global problem until now and successful treatment has not yet been discovered (Malviya *et al.*, 2010). Currently available therapy for diabetes includes insulin and various oral hypoglycemic agents such as sulfonylureas, metformin, glucosidase inhibitors, troglitazone, and others. But these conventional therapies are reported to produce serious adverse side effects such as liver problems, lactic acidosis and diarrhea (Rajalakshmi *et al.*, 2009). There is therefore an emerging increase in the consumption of herbal formulations by the public because of the strong belief that these products are natural; hence, they are safe for the treatment of ailments. However, herbal preparations which may be assumed to be safe may contain toxins and its long term use may cause nephrotoxicity, hepatotoxicity, cardiotoxicity, neurotoxicity and skin toxicity (Fatima & Nayeem, 2016). For instance, *Maerua decumbens* leaves have been reported to be toxic since they are usually used to poison fish during fishing expeditions by many

communities (Quattrocchi, 2012), where the leaves are pound into small pieces and thrown into the water.

1.3 Justification

Herbal medicines are preferred because of ease in availability, rising cost of medical care, low cost, potency and efficiency, enhanced tolerance, perceived fewer side effects, complete accessibility and recyclability (Maiti *et al.*, 2011). The use of *Maerua decumbens* roots in this study was based on the claim that it is traditionally used to treat diabetes (Kigen *et al.,* 2014) and the fact that its leaves are used as poison in traditional fishing expeditions (Ghazanfar, 1994). However, there is no scientific documentation on the above claims of antidiabetic activity and the safety hence the justifications of the current study. The whole plant, crude extracts or purified constituents are used in indigenous system of medicines, which have ultimately evolved into modern therapeutic sciences (Hamayun *et al*., 2003). In this study, the crude extracts were used since the communities mostly use boiled concoctions for treatment. Experimental screening of the toxicity of these plants is crucial to assure the safety and effectiveness of those natural sources, prior to human exposure. Based on historical research, the oral route administration is the most convenient and commonly used one when studying toxicity. The herbals of the roots are taken orally and also justifies why the administration is via the oral route. Rodents act as the best choice of use in research due to smaller size, easy to handle, omnivorous in nature, and non-wild tranquil behavior. Wistar albino rats are preferred to genetic models due to lower cost, wider availability, easier to induce diabetes and to maintain compared to genetic models. They also possess all the major pathogenesis of the disease as it is usually found in humans (Wilson & Islam, 2015). The

Organization for Economic Cooperation and Development guidelines are used during acute and sub-acute oral toxicity testing (OECD, 1995). The guidelines provide for the justifiable use of such parameters as body and organ weights, hematological, biochemical and histopathology to assess toxicity of chemical substances.

Diabetes is increasingly affecting a growing number of patients and seriously reducing their quality of life. Use of conventional drugs in diabetes management is expensive, thus, unaffordable to most patients. Furthermore most of these conventional drugs are associated with undesirable side effects. Incorporation of herbal medicine into conventional healthcare system may significantly improve the overall healthcare system. The main parameters mostly affected during diabetic state include body weight and blood glucose levels which were are assessed in this study. Also, liver malondialdehyde (MDA) level a marker of oxidative stress was assessed in the diabetic rats, following treatments with the *M. decumbens* root extract (WHO, 2016).

The streptozotocin (STZ)-induced Wistar rat model for type 1 diabetes is a good model that has been used extensively in animal models to study both the pathology of diabetes mellitus and complications related to the disease as well as possible interventions (Furman, 2015). Streptozotocin (STZ) is well known for its selective pancreatic islet cell toxicity and has been extensively used for the induction of diabetes mellitus in animals (Furman, 2015). Previously reported literature indicates that the type of diabetes and characteristics differ with the employed dose of STZ and animal or species used (Rochette *et al.*, 2014). STZ induced diabetes provides a relevant example of endogenous chronic oxidative stress due to the resulting hyperglycemia, which has been shown to

induce free radical production and cause tissue injury. Phytochemicals analysis will be done to identify presence of antidiabetic agents (Murugan *et al.*, 2013).

1.4 Study Objectives

1.4.1 General Objective

The general objective of this study was to determine the antidiabetic activity and safety of methanolic root extract of *Maerua decumbens* in Wistar albino rats.

1.4.2 Specific Objectives

The specific objectives of this study were;

1. To qualitatively determine phytochemical composition of the methanolic root extract of *Maerua decumbens.*
2. To evaluate the safety of orally administered doses of the methanolic root extract of *M. decumbens*, on the body and organ weights, serum indices of liver and kidney function, hematological parameters and tissue histology in Wistar albino rats.
3. To determine Antidiabetic activities of orally administered methanolic root extract of *M. decumbens*, on body weight, fasting blood sugar, liver lipid peroxidation and tissue histology in STZ-induced diabetic Wistar albino rats.

1.5 Null Hypotheses

H_{01}: There are no phytochemical constituents present in methanolic root extract of *Maerua decumbens.*

H_{02}: Oral administration of methanolic root extract of *Maerua decumbens* is not toxic when orally administered to Wistar albino rats.

H_{03}: Methanolic root extract of *Maerua decumbens* does not possess antidiabetic activity in STZ-induced diabetic Wistar albino rats.

1.6. Overall Study Significance

This research was expected to provide scientific basis of the use of *Maerua decumbens* root extracts as a potential antidiabetic herbal remedy. The information obtained from its safety study may also assist in making decisions on its continued use in treatment of diabetes mellitus and treatment of other ailments traditionally. The knowledge generated from the study was to stimulate further research on other pharmacological properties of *Maerua decumbens*, which were beyond the scope of this study. The study was also to aid in preservation of knowledge on medicinal value of *M. decumbens* which is currently scanty.

CHAPTER TWO

LITERATURE REVIEW

2.1 Diabetes Mellitus

Diabetes mellitus is a metabolic disorder of multiple aetiology characterised by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism; resulting from defects in insulin secretion, insulin action, or both (ADA, 2010). Characteristic clinical features of the disease include glycosuria, polyuria, and polydipsia. Glucose spill-over in urine (glycosuria) occurs when renal threshold for glucose reabsorption is exceeded. This causes osmotic diuresis (polyuria), which results in increased dehydration and thirst, with increased drinking or polydipsia (WHO, 2013). The two most common types of diabetes mellitus are IDDM or (type 1) and NIDDM or (type 2). Weight loss in type 1 diabetes mellitus also occurs due to an increased breakdown and reduced synthesis of proteins. In the absence of insulin in acute conditions, diabetic ketoacidosis results and can lead to stupor, coma, and death. Chronic uncontrolled hyperglycemia progressively leads to the development of complications such as retinopathy, neuropathy, macro- and micro vascular diseases (ADA, 2010).

2.2 Types of Diabetes Mellitus

WHO classification of diabetes introduced in 1980 and revised in 1985 was based on clinical characteristics. The two most common types of diabetes are IDDM or (type 1) and NIDDM or (type 2). WHO classification also recognized malnutrition-related diabetes mellitus and gestational diabetes as other types of diabetes mellitus.

Malnutrition-related diabetes was omitted from the new classification because its etiology is uncertain, and it is unclear whether it is a separate type of diabetes (Holt, 2004).

2.2.1 Type 1 Diabetes Mellitus

Type 1 diabetes represents approximately 10% of all cases of diabetes and develops secondary to autoimmune destruction of the insulin-producing β-cells of the pancreas. Due to the pathophysiology, insulin therapy is indicated at the onset of this disease. Type 1 diabetes represents a heterogenous and polygenic disorder, with a number of non-Human Leukocyte Antigen loci contributing to disease susceptibility (Bluestone *et al*., 2010). There is yet no identified agent substantially capable of preventing this type of disease (Bluestone *et al*., 2010).

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

A strong association has also been drawn between type 1 diabetes and human leukocyte antigen, with linkage to the *DQA* and *DQB* genes, and it is influenced by the *DRB* genes. These

HLA-DR/DQ alleles can be either predisposing or protective (Concannon *et al*., 2009). In this form of diabetes, the rate of β-cell destruction is quite variable, being rapid mainly in infants and children and slow mainly in adults. Some patients, particularly children and adolescents, may present with ketoacidosis as the first manifestation of the disease. Others have modest fasting hyperglycemia that can rapidly change to severe hyperglycemia and/or ketoacidosis in the presence of infection or other stresses. Still others, particularly adults, may retain residual β-cell function sufficient to prevent ketoacidosis for many years; such individuals eventually become dependent on insulin for survival and are at risk for ketoacidosis (ADA, 2014). At this latter stage of the disease, there is little or no insulin secretion, as manifested by low or undetectable levels of plasma C-peptide (ADA, 2010). Autoimmune destruction of β-cells has multiple genetic predispositions and is also related to environmental factors that are still poorly defined. Although patients are rarely obese when they present with this type of diabetes, the presence of obesity is not incompatible with the diagnosis (ADA, 2010).

2.2.2 Type 2 Diabetes Mellitus

This form of diabetes accounts for 90–95% of people with diabetes. Type 2 diabetes results from a combination of defects in insulin secretion and insulin action, either of which may predominate (WHO, 1999). At least initially, and often throughout their lifetime, these individuals with type

2 diabetes do not need insulin treatment to survive, but may require it for the control of blood glucose levels if this is not achieved with diet alone or with oral hypoglycemic agents. Type 2 diabetes is a progressive disorder, which is associated with diminishing pancreatic function over time. Recognition of this phase is important in the clinical management of the disorder because depending on the stage, effective control may require lifestyle modification, oral agent therapy, oral agents combined with insulin, or insulin alone. Insulin resistance, which is defined as a clinical state in which a normal or elevated insulin level produces an inadequate biological response, is considered to be a hallmark for the presence of metabolic syndrome and type 2 diabetes mellitus (Cefalu, 2006).

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

Although the specific etiologies are not known, autoimmune destruction of β-cells does not occur in type 2 diabetes. The risk of developing this form of diabetes increases with age, obesity and lack of physical activity. It occurs more frequently in women with prior gestational diabetes mellitus and in individuals with hypertension or dyslipidemia, and its

frequency varies in different racial or ethnic subgroups (Defronzo, 2009), for instance African Americans have higher risk since these populations are more likely to be overweight or high blood pressure It is often associated with a strong genetic predisposition, more so than is the autoimmune form of type 1 diabetes mellitus. However, the genetics of this form of diabetes are complex and not clearly defined (ADA, 2010). Patients who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region **(**Tripathi & Srivastava, 2006).

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

2.2.3 Gestational Diabetes Mellitus

Gestational diabetes (GDM) is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. A steady decline in insulin sensitivity as gestation progresses is a normal feature of pregnancy; gestational diabetes results when maternal insulin secretion cannot increase sufficiently to counteract the decrease in insulin

sensitivity (Buchanan *et al*., 2012). Approximately 7% of all pregnancies are complicated by GDM, resulting in more than 200,000 cases annually (Buchanan *et al*., 2012). Clinical characteristic risk factors of GDM include; marked obesity, personal history of GDM, glycosuria, or a strong family history of diabetes (ADA, 2004).

2.3 Causes of Diabetes Mellitus

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

2.3.1 Causes of Type 1 Diabetes Mellitus

Type 1 diabetes mellitus has a genetic component which must be present for susceptibility to occur. The exact mechanism is unclear but transmission is believed to be autosomal dominant, recessive or mixed. If a first-degree relative has the disease, the child has a 5-10 % chance of developing type 1 diabetes (Blackburn, 2014). The susceptibility gene resides on the short arm on the sixth chromosome, either within or in close proximity to the major histocompatibility complex, that is, the HLA region (Barrett *et al.,* 2009).

Nutrition also leads to development of type 1 diabetes mellitus. Nutrition given during the neonatal period and early infancy including dietary factors such as neonatal exposure to Bovine serum antigen (BSA) in cow's milk and chemical toxins in food and stress initiates injury to pancreatic β-cells (Patelarou *et al.,* 2012). In addition, exposure to

viruses and allergens or both initiates the process in genetically susceptible individuals. This external influence precipitates an inflammatory response in the pancreas known as insulitis. Activated T-lymphocytes infiltrate the islet cells in the pancreas. Macrophages and T-cells lead to β-cell destruction via localized release of cytokines (Ting *et al.*, 2013). Cytotoxic amounts of nitric oxide and reactive oxygen intermediates are also released, contributing to free radical damage to the β-cells (Richer & Horwitz, 2008).

Some viruses seem to attack and destroy the β-cells directly. For example, exposure to enterovirus infections either in uterus or during childhood may initiate β-cell damage and subsequent type 1 diabetes mellitus. Autoimmune destruction of insulin producing pancreatic β-cells, leading to an absolute deficiency in insulin synthesis and secretion may also lead to type 1 diabetes mellitus. The autoimmune β-cell destruction may persist over a prolonged period prior to diagnosis of the disease, but loss of β-cell mass often accelerates markedly about 6 months before clinical presentations (Eizirik *et al.*, 2009). Moreover, several cytokines are expressed in type 1 diabetes mellitus and the pattern of the network in which these cytokines cooperate is very complex (Rabinovitch & Suarez-Pinzon, 2007). Cytokines are small polypeptides and like hormones, they act as messengers between cells in order to control the immune system, inflammation, cell growth and hematopoiesis. Most cytokines act locally, but some such as IL-1β, IL-6 and TNF-α may also have systemic effects. A cytokine is often produced not only in immune cells but also in other cell types such as stromal cells and epithelial cells. Paradoxically, part of the β-cell destruction might be caused by cytokines produced by the β-cell themselves (Rabinovitch & Suarez-Pinzon, 2007). Similarly, type 1 T helper (Th1) and type 2 T helper (Th2) cells have also been suggested to be involved in the destruction of

cells responsible for insulin formation. An increased expression of Th1 cytokines correlates with β-cell destruction and diabetes development while the expression of Th2 cytokines correlates with benign, non-destructive insulitis characterized by an immune cell infiltration around the islets without significant loss of β-cells (Eizirik & Mandrup-Poulsen, 2001).

2.3.2 Causes of type 2 Diabetes Mellitus

Type 2 diabetes mellitus shows strong familial aggression. Twins and family studies have shown firm evidence that the role of the genetic component is relatively strong. Several genes have been suggested as markers for type 2 but apart from evidence for abnormalities in the adenosine deaminase and glucokinase genes, no other consistent abnormalities have yet been found (Sladek *et al*., 2007). Nutrition has a role to play in type 2 diabetes. Increased intake of saturated fats and decreased intake of dietary fiber can result in a decrease of insulin sensitivity and abnormal glucose tolerance. This diet is also accompanied by other changes such as arterial hypertension, dislipidaemia and obesity (De Souza *et al*., 2005). Severe or prolonged stress/trauma are associated with glucose intolerance induced by hormonal effects on glucose metabolism and insulin secretion and action but this remains unproven (Surwit *et al.*, 2002). Drugs have been indicated to interfere with the process of glucose metabolism such as phenytoin, diuretics corticosteroids, some contraceptive steroids and β-adrenoreceptor antagonists' agents, which may cause glucose intolerance and in susceptible individuals, may induce diabetes and usually resolves after withdrawal of the drug (Wu *et al.*, 2014).

2.4 Epidemiology of Diabetes Mellitus

2.4.1 Global Prevalence of Diabetes Mellitus

The rates of diabetes cases continue to increase globally. Diabetes mellitus is a serious condition with potentially devastating complications that affects all age groups worldwide. In 1985, an estimated 30 million people around the world had been diagnosed with diabetes; in 2000, the figure rose to over 150 million; and, in 2012, the International Diabetes Federation (IDF) estimated that 371 million people had diabetes. This number is projected to rise to about 552 million (that is 1 in 10 adults) by 2030, which is equivalent to 3 new cases per second (IDF, 2013).

There are marked differences in prevalence of diabetes between developed and developing countries. Figure 2.1 shows the current estimated numbers of people with diabetes by age-group for 2010 and projected in 2030. For developing countries, adult diabetes numbers are likely to increase by 69% from 2010 to 2030, compared to 20% for developed countries, whereas total adult populations are expected to increase by 36% and 2% respectively. For the developing countries, increases in diabetes numbers are expected for each age-group, with a doubling for the over 60-year age-group. For developed countries, an increase of 38% is only expected amongst those over 60s, with slight decreases predicted for the younger age-groups. Currently, the greatest number of people worldwide with diabetes is in the 40–59 year-old age-group, but by 2030, there will be slightly more people with diabetes in the 60–79 year-old age-group (Guariguata *et al.*, 2014), due to increased urbanization, sedentary lifestyles among other factors. The overall total predicted increase in numbers with diabetes from 2010 to 2030 is 54%, at an

annual growth of 2.2%, which is nearly twice the annual growth of the total world adult population (Figure 2.1).

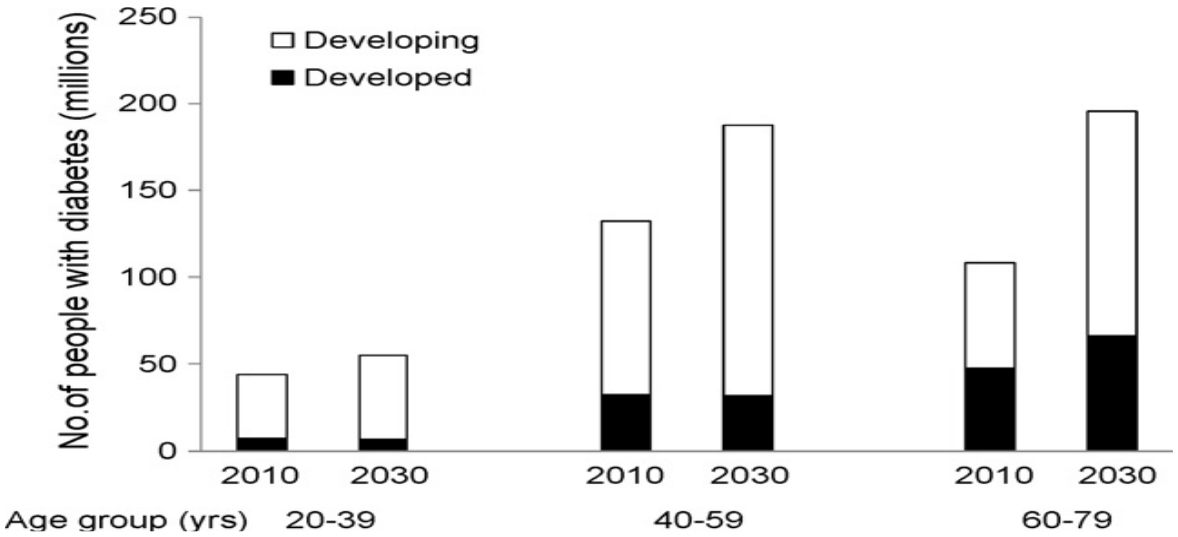

Figure 2.1: Global Diabetes in 2010 and 2030 by age group (Guariguata *et al.*, 2014)

World diabetes day is observed annually on the 14th day of November globally. It serves to remind governments of their commitment to develop national policies for diabetes prevention, treatment and care, a pledge many countries made during the 2011 United Nations (UN) high level meeting on non-communicable diseases (Guariguata *et al.*, 2014).

2.4.2 Prevalence of Diabetes Mellitus in Kenya

The World Health Organization estimates that the prevalence of diabetes in Kenya is at 3.3% (Chege, 2010) and it is predicted to rise to 4.5% by 2025 (Mcferran, 2008). However, two-thirds of diabetics are undiagnosed (Beran & Yudkin, 2006). In Kenya a recent study has shown a prevalence of 4.2% in the general population, with a prevalence of 2.2% in the rural areas and a prevalence of 12.2% in the urban population (Christensen

et al., 2009), partly due sedentary lifestyles. The number of people with diabetes is increasing due to population growth, aging, urbanization and increasing prevalence of obesity and physical inactivity (Wild *et al.*, 2004). In 2014, the prevalence of diabetes in adults in Kenya was 3.6% out of an adult population of about 21,556,000 (Guariguata *et al.*, 2014) as shown in Figure 2.2. Many middle- and low-income countries have more people under the age of 60 with diabetes compared to the world average. Meanwhile, for high-income countries, a growing population over the age of 60 makes up the largest proportion of diabetes prevalence. A study has showed that neighborhoods with high levels of poverty are associated with increases in the incidence of extreme obesity and diabetes (Ludwig *et al.*, 2011).

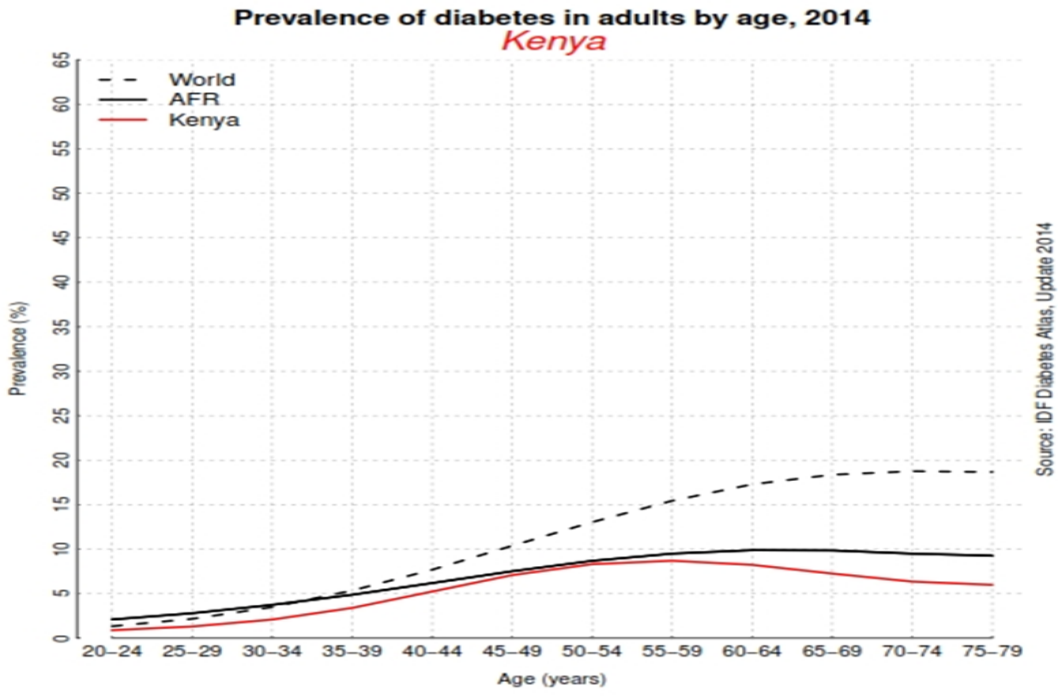

Figure 2.2: Prevalence of diabetes in Kenya by age (Guariguata *et al.*, 2014). *AFR-Africa*
The dotted line is the distribution of diabetes prevalence by age for the world; the black line is the distribution for the Africa region; and the country (Kenya) distribution is plotted in the red line.

2.4.3 Mortality

Mortality from Type 1 diabetes is mainly due to long-term complications and mortality caused by onset ketoacidosis or diabetic ketoacidosis after onset, although part of the excess mortality has been unexplained deaths in bed (Dahlquist & Källén, 2005). Type 2 diabetes is associated with excess mortality which is mainly attributed to the vascular complications of the disease. The excess mortality leads to a decreased life expectancy among patients with type 2 diabetes. The extent of the reduction of life expectancy is dependent on the age of onset of the disease, but it averages approximately 10 years. Increased mortality in patients with type 2 diabetes is seen especially among those with diabetic complications (Grover *et al.*, 2015). Risk factors include proteinuria and retinal disease, and the classical risk factors for heart disease. Hyperlipidaemia, hypertension and smoking, each contribute disproportionately to the death rates among those with type 2 diabetes mellitus, and the rates of mortality also increase with increasing duration of the disease (Steyn *et al.*, 2004).

2.5 Risk Factors of Diabetes Mellitus

The risk factors for type 1 diabetes are still being researched. However, having a family member with type 1 diabetes slightly increases the risk of developing the disease. Environmental factors and exposure to some viral infections have also been linked to the risk of developing type 1 diabetes. According to (Ekoé *et al.*, 2013), the major risk factors for type 2 diabetes include the following: Family history of diabetes, overweight, unhealthy diet, physical inactivity, increasing age, high blood pressure, ethnicity, history of gestational diabetes and poor nutrition during pregnancy. Other risk factors include;

depression (Pan *et al.*, 2010) and schizophrenia (Siuta *et al.*, 2010). The major risk factors of diabetes mellitus are discussed in the subsequent subscections.

2.5.1 Genetic Factors

The causes of type 1 diabetes are unknown, although several risk factors have been identified. The risk of developing type 1 diabetes mellitus is increased by certain variants of the *HLA-DQA1, HLA-DQB1,* and *HLA-DRB1* genes. These genes provide instructions for making proteins that play a critical role in the immune system (Howson *et al.*, 2009). The *HLA-DQA1, HLA-DQB1,* and *HLA-DRB1* genes belong to a family of genes called the human leukocyte antigen (HLA) complex (Barrett *et al.*, 2009). The HLA complex helps the immune system distinguish the body's own proteins from proteins made by foreign invaders such as viruses and bacteria. Type 1 diabetes is generally considered to be an autoimmune disorder. Autoimmune disorders occur when the immune system attacks the body's own tissues and organs. For unknown reasons, in people with type 1 diabetes mellitus, the immune system damages the insulin-producing beta cells in the pancreas. Damage to these cells impairs insulin production and leads to the signs and symptoms of type 1 diabetes mellitus.

Certain HLA haplotypes are associated with a higher risk of developing type 1 diabetes mellitus, with particular combinations of *HLA-DQA1*, *HLA-DQB1*, and *HLA-DRB1* gene variations resulting in the highest risk (Kelly *et al*., 2003). These haplotypes seem to increase the risk of an inappropriate immune response to beta cells. However, these variants are also found in the general population, and only about 5 percent of individuals with the gene variants develop type 1 diabetes mellitus. HLA variations account for approximately 40 percent of the genetic risk for the condition. Other HLA variations

appear to be protective against the disease. Additional contributors, such as environmental factors and variations in other genes, are also thought to influence the development of this complex disorder (Bluestone *et al*., 2010).

The genetics of type 2 diabetes are complex and are not completely understood. Evidence supports the involvement of multiple genes in pancreatic beta-cell failure and insulin resistance. Genome-wide association studies have identified dozens of common genetic variants associated with increased risk for type 2 diabetes (Billings & Florez, 2010). Among the variants thus far discovered, the one with the strongest effect on susceptibility is the transcription factor 7–like 2 (*TCF7L2*) gene. These identified genetic variants account for almost 10% of the hereditary cases mostly in type 2 diabetes mellitus (Billings & Florez, 2010).

Some forms of diabetes have a clear association with genetic defects. The syndrome that is historically referred as maturity onset diabetes of the young (MODY), now understood to be a variety of defects in beta-cell function, accounts for 2-5% of individuals with type 2 diabetes who present at a young age and have mild disease. MODY is a group of monogenic disorders characterized by autosomal dominantly inherited non-insulin dependent form of diabetes classically presenting in adolescence or young adults before the age of 25 years (Siddiqui *et al*., 2015). MODY is a rare cause of diabetes (1% of all cases) and is frequently misdiagnosed as Type 1 diabetes or Type 2 diabetes. A precise molecular diagnosis is essential because it leads to optimal treatment of the patients and allows early diagnosis for their asymptomatic family members. Mutations in the glucokinase (GCK) and hepatocyte nuclear factor (HNF) 1A/4A genes are the most common causes of MODY. GCK mutations cause a mild, asymptomatic, and stable

fasting hyperglycemia usually requiring no specific treatment (Siddiqui *et al*., 2015). However, mutations in the HNF1A and HNF4A cause a progressive pancreatic β-cell dysfunction and hyperglycemia that can result in microvascular complications (Anik *et al*., 2015).

2.5.2 Diet

Type 1 diabetes mellitus is an immune-mediated disease which is characterized by a preclinical prodrome during which β cell autoimmunity proceeds at a variable rate. Breastfeeding, nicotinamide, zinc, and vitamins C, D, and E is reported as possibly protecting against type 1 diabetes, whereas *N*-nitroso compounds, cow milk, increased linear growth, and obesity may increase the risk to type 1 diabetes (Virtanen & Knip, 2003). On the other hand, the findings in a review by (Hu *et al.*, 2001), indicate that neither total fat nor total carbohydrate as proportions of total energy play a major part in the development of type 2 diabetes in humans but the different types of fat and carbohydrates appear to be more important. In particular, a higher intake of polyunsaturated fat and long-chain Ω-3 fatty acids could be beneficial, whereas a higher intake of saturated fat and trans-fat could adversely affect glucose metabolism (Riccardi *et al*., 2004).

In dietary practice, exchanging non-hydrogenated polyunsaturated fat for saturated and *trans*-fatty acids could appreciably reduce risk of type 2 diabetes. In addition, a low glycaemic index diet with a greater amount of fiber and minimally processed whole grain products has been shown to improve glycaemic and insulinaemic responses and lower the risk of type 2 diabetes (Venkasetan & Sengupta, 2015). From existing evidence it is therefore possible that omega-3 fatty acids, low glycemic index foods and exclusive

breastfeeding may play a protective role, and that total fat intake and *trans*-fatty acids may contribute to the risk. It is recommended that a normal weight status in the lower BMI range (BMI 21–23) be maintained and that saturated fat intake is less than 7% of the total energy intake (Steyn *et al.*, 2004).

2.5.3 Physical Inactivity

As the number of people diagnosed with diabetes continues to grow, researchers are focusing on discovering why the prevalence of the disease is increasing. New research has found out that ceasing regular physical activity impairs glucose control; suggesting that inactivity may play a key role in the development of type 2 diabetes mellitus. Physical inactivity has been identified as the fourth leading risk factor for 6% global mortality (WHO, 2016). Moreover, physical inactivity is estimated to be the main cause for approximately 21–25% of breast and colon cancers, 27% of diabetes and approximately 30% of ischemic heart disease burden (Booth *et al*., 2012). Regular and adequate levels of physical activity in adults reduce the risk of hypertension, coronary heart disease, stroke, diabetes, breast and colon cancer, depression and weight control (Samuelson, 2004).

2.6 Pathophysiology of Diabetes Mellitus

The pancreas plays a primary role in the metabolism of glucose by producing and secreting the hormones like insulin and glucagon. Insulin is a protein that is essential for proper regulation of glucose and for maintenance of proper blood glucose levels (Worthley, 2003). Glucagon is a hormone that opposes the action of insulin. It is secreted when blood glucose level falls. It increases blood glucose concentration, partly by

stimulating the breaking down of stored glycogen in the liver by a pathway known as glycogenolysis and by gluconeogenesis in the liver which is the production of glucose from non-carbohydrate precursors such as glycogenic amino acids (Conn & Stumpf, 2009).

Type 1 diabetes mellitus is characterized by the loss of insulin secretion due to autoimmune destruction of pancreatic beta cells and a resultant ketoacidosis which requires insulin replacement for survival. Genetic, environmental and may be other unknown factors may contribute to the disease susceptibility. The DR3/DR4 alleles in the MHC (HLA) complex have been clearly implicated in the disease risk (Jabbour & Stephens, 2007).

Type 2 diabetes is characterized by a combination of peripheral insulin resistance and inadequate insulin secretion by pancreatic beta cells. Insulin resistance, which has been attributed to elevated levels of free fatty acids and proinflammatory cytokines in plasma, leads to decreased glucose transport into muscle cells, elevated hepatic glucose production, and increased breakdown of fat (Morino *et al.*, 2006). A role for excess glucagon production cannot be underestimated; indeed, type 2 diabetes is an islet paracrinopathy in which the reciprocal relationship between the glucagon-secreting alpha cell and the insulin-secreting beta cell is lost, leading to hyperglucagonemia and hence the consequent hyperglycemia (Unger & Orci, 2010). For type 2 diabetes to occur, both insulin resistance and inadequate insulin secretion must exist. For instance, all overweight individuals have insulin resistance, but diabetes develops only in those who cannot increase insulin secretion sufficiently to compensate for their insulin resistance. Their insulin concentrations may be high, yet inappropriately low to counteract the high

level of hyperglycaemia (Unger & Orci, 2010). A simplified scheme for the pathophysiology of abnormal glucose metabolism in type 2 diabetes mellitus is depicted in Figure 2.3.

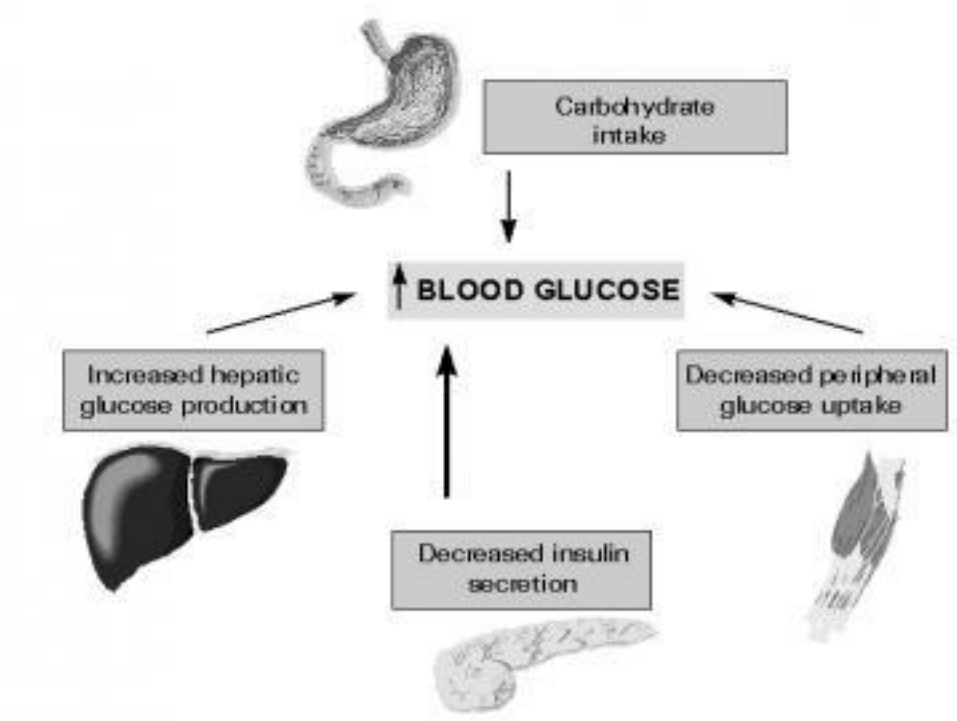

Figure 2.3: Simplified scheme for the pathophysiology of type 2 diabetes (Unger & Orci, 2010).

2.6.1 Oxidative Stress in Diabetes Mellitus

It is believed that oxidative stress plays important role in the development of vascular complications in diabetes particularly type 2 diabetes mellitus. Reactive oxygen species (ROS) levels increase in diabetes and may be due to decrease in the production of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH–Px) (Asmat *et al*., 2016). Nowadays, evidences have been reported that support the role of oxidative stress in the pathogenesis of both type 1 and

type 2 diabetes. Free radical formation in diabetes by non-enzymatic glycation of proteins, glucose oxidation and increased lipid peroxidation leads to damage of enzymes, cellular machinery and also increased insulin resistance due to oxidative stress. According to latest research, lipid is not only but also the apolipoprotein component of low density lipoprotein that forms insoluble aggregates oxidatively due to hydroxyl radical- induced cross-linkage between apo-B monomers that is responsible for oxidative damage in diabetic complications (Matough *et al.,* 2012). In diabetes mellitus, main sources of oxidative stress are in mitochondria (Asmat *et al.*, 2016). During oxidative metabolism in mitochondria, a component of the utilized oxygen is reduced to water, and the remaining oxygen is transformed to oxygen free radical ($O_{\cdot}$) which is an important ROS that is converted to other radical scavenger such as $ONOO_{\cdot}$, OH and H_2O_2 (Asmat *et al.,* 2016).

Many evidences from experiments have given a link between diabetes and oxidative stress by measuring various biomarkers that include DNA damage biomarkers and lipid peroxidation products. It is believed that in the onset and progression of late diabetic complication, free radicals have a major role due to their ability to damage lipids, proteins and DNA. Free radical and oxidative stress induced complications from diabetes mellitus include coronary artery disease, neuropathy, nephropathy, and retinopathy and stroke (Ullah *et al.,* 2015). Diabetes mellitus produces disturbances in the lipid profile of the body making the cells more susceptible to lipid peroxidation (Pérez-Matute *et al*., 2009). Experimental studies show that polyunsaturated fatty acids in cell membrane are extremely prone to attack by free radicals due to the presence of multiple bonds. Lipid hyperperoxides (LHP) through intermediate radical reactions produce such fatty acids

that generate highly reactive and toxic lipid radicals that form new LHP (Matough *et al*., 2012). A critical biomarker of oxidative stress is lipid peroxidation which is the most explored area of research when it comes to ROS. Malondialdehyde (MDA) is formed as a result of lipid peroxidation that can be used to measure lipid peroxides after reacting it with thiobarbituric acid (Ullah *et al.,* 2015) and the colour formed is determined spectrophotometrically.

2.6.2 Beta-Cell Dysfunction

Beta cell insults include cytokine-induced inflammation, obesity and insulin resistance, and overconsumption of saturated fat and free fatty acids (FFA). A progressive decline of beta cell function leading to beta cell exhaustion precedes beta cell demise. Loss of beta cell mass and function are central to the development of both type 1 and 2 diabetes mellitus (Talchai *et al.,* 2012). Beta-cell dysfunction is a major factor across the spectrum of prediabetes to diabetes. A study of obese adolescents done by Bacha *et al.*, (2010) confirms what is increasingly being stressed in adults as well, that beta-cell dysfunction develops early in the pathologic process and does not necessarily follow the stage of insulin resistance. Singular focus on insulin resistance as the "be all and end all" is gradually shifting, and hopefully better treatment options that address the beta-cell pathology will emerge for early therapy (Bacha *et al*., 2010).

2.6.3 Insulin Resistance

Insulin resistance is when cells of the body don’t respond properly to the hormone insulin. Insulin resistance is the driving factor that leads to type 2 diabetes, gestational diabetes and prediabetes. The role of insulin is to allow cells of the body to take in

glucose to be used as fuel or stored as body fat. It also means that glucose is more likely to build up in the blood and this can lead to too high blood sugar levels. When the body becomes resistant to insulin, it tries to cope by producing more insulin. People with insulin resistance are often producing too more insulin than healthy people. Producing too much insulin is known as hyperinsulinemia (Fu *et al.*, 2013).

One of insulin's functions is to regulate delivery of glucose into cells to provide them with energy. Insulin resistant cells cannot take in glucose, amino acids and fatty acids. Thus, glucose, fatty acids and amino acids 'leak' out of the cells. A decrease in insulin/glucagon ratio inhibits glycolysis which in turn decreases energy production. The resulting increase in blood glucose may raise levels outside the normal range and cause adverse health effects. Certain cell types such as fat and muscle cells require insulin to absorb glucose. When these cells fail to respond adequately to circulating insulin, blood glucose levels rise (Ghasemi *et al*., 2013). The liver helps regulate glucose levels by reducing its secretion of glucose in the presence of insulin. This normal reduction in the liver's glucose production may not occur in people with insulin resistance. Insulin resistance in muscle and fat cells reduces glucose uptake and storage whereas insulin resistance in liver cells results in reduced glycogen synthesis and storage and also a failure to suppress glucose production and release into the blood stream.

Increased mobilization of stored lipids in these cells elevates free fatty acids in the blood plasma. Elevated blood fatty-acid concentrations, reduced muscle glucose uptake, and increased liver glucose production all contribute to elevated blood glucose levels (Saltiel & Kahn, 2001). High plasma levels of insulin and glucose due to insulin resistance are a major component of the metabolic syndrome. If insulin resistance exists, more insulin

needs to be secreted by the pancreas. If this compensatory increase does not occur, blood glucose concentrations increase and type 2 diabetes mellitus occurs. Any food or drink containing glucose causes blood glucose levels to increase. In normal metabolism, the elevated blood glucose level instructs beta cells in the Islets of Langerhans, located in the pancreas, to release insulin into the blood. The insulin, in turn, makes insulin-sensitive tissues in the body (primarily skeletal muscle cells, adipose tissue, and liver) absorb glucose, and thereby lower the blood glucose level (Leibiger *et al*., 2008). The beta cells reduce insulin output as the blood glucose level falls, allowing blood glucose to settle at a constant level of approximately 5 mmol/l. The most common type of insulin resistance is associated with overweight and obesity in a condition known as the metabolic syndrome. Insulin resistance often progresses to full Type 2 diabetes mellitus.

Various disease states make body tissues more resistant to the actions of insulin. Examples include infection (mediated by the cytokine TNFα) and acidosis. Certain drugs also may be associated with insulin resistance (e.g., glucocorticoids) (Guilherme *et al*., 2008). The presence of insulin leads to a kind of insulin resistance; every time a cell is exposed to insulin, the production of GLUT4 (Glucose transporter type 4) on the membrane of the cell decreases somewhat. In the presence of a higher than usual level of insulin, this down-regulation acts as a kind of positive feedback, increasing the need for insulin.

Elevated blood levels of glucose lead to increased glycation of proteins with changes. Insulin resistance often is found in people with visceral adiposity, hypertension, hyperglycemia, and dyslipidemia involving elevated triglycerides, small dense low-density lipoprotein (sdLDL) particles, and decreased High Density Lipoprotein (HDL)

cholesterol levels. With respect to visceral adiposity, a great deal of evidence suggests two strong links with insulin resistance. First, unlike subcutaneous adipose tissue, visceral adipose cells produce significant amounts of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-a), and Interleukins-1 and -6, and others (Eckel *et al*., 2005). Much of the attention on production of proinflammatory cytokines has focused on the IKK-beta/NF-kappa-B pathway, a protein network that enhances transcription of inflammatory markers and mediators that may cause insulin resistance. Second, visceral adiposity is related to an accumulation of fat in the liver, a condition known as non-alcoholic fatty liver disease (NAFLD). The result of NAFLD is an excessive release of free fatty acids into the bloodstream due to increased lipolysis, and an increase in hepatic glycogenolysis and hepatic glucose production, both of which have the effect of exacerbating peripheral insulin resistance and increasing the likelihood of Type 2 diabetes mellitus (Zeng *et al*., 2015).

The facilitative transport of glucose across animal cell membranes is mediated by members of the Glut protein family. These 50- to 60-kDa glycoproteins are ubiquitously expressed in mammalian tissues and are responsible for the uptake of sugar from the blood into cells, supplying cellular glucose for adenosine triphosphate (ATP) production and for a wide variety of anabolic reactions. Additionally, glucose transport in certain tissues plays a critical role in whole-body glucose homeostasis. During fasting or starvation, the liver and kidney maintain euglycemia by becoming net producers of blood glucose, initially via the breakdown of glycogen stores and later through the catabolism of amino acids (Mueckler, 2001).

The appropriate output or uptake of glucose from these tissues via the Glut proteins is critical to health and survival. For example, a disruption in the normal process by which insulin stimulates glucose transport into skeletal muscle is the cause of the peripheral insulin resistance associated with NIDDM. Insulin resistance is the earliest detectable abnormality observed during the natural history of NIDDM and is believed to be a major triggering event in the development of the disease. Thus, a detailed understanding of how insulin regulates glucose transport in muscle is essential to unraveling the molecular basis of NIDDM (Huang & Czech, 2007).

2.6.4 Genomic Factors

Genome wide association studies of single nucleotide polymorphisms (SNPs) have identified a number of genetic variants that are associated with β-cell function and insulin resistance. Some of the SNPs appear to increase the risk for type 2 diabetes. Over forty independent loci which demonstrate their associations with an increased risk for type 2 diabetes have been identified (Wheeler & Barroso, 2011) of which the subsets of the most potent ones and their associated effects are listed below (Billings & Florez, 2010):-

- Decreased beta-cell responsiveness, leading to impaired insulin processing and decreased insulin secretion (*TCF7L2)*
- Lowered early glucose-stimulated insulin release (*MTNR1B, FADS1, DGKB, GCK*)
- Altered metabolism of unsaturated fatty acids (*FSADS1*)
- Dysregulation of fat metabolism (*PPARG*)
- Inhibition of serum glucose release (*KCNJ11*).

- Increased adiposity and insulin resistance (*FTO* and *IGF2BP2*).
- Control of the development of pancreatic structures, including beta-islet cells (*HHEX*).
- Transport of zinc into the beta-islet cells, which influences the production and secretion of insulin (*SLC30A8*).
- Survival and function of beta-islet cells (*WFS1*).

Susceptibility to type 2 diabetes mellitus is also associated with genetic variants involving incretin hormones which are released from endocrine cells in the gut and which stimulate insulin secretion in response to digestion of food. For instance, reduced beta-cell function has been associated with a variant in the gene that codes for the receptor of gastric inhibitory polypeptide (*GIPR*) (Saxena *et al.*, 2010).

2.6.5 Diabetic Dyslipidemia

Dysregulation of lipid metabolism is a key feature of some pathological conditions including diabetes mellitus, insulin resistance, obesity, and fatty liver (Amer & Langin, 2014). In diabetes, a spectrum of abnormalities including increased serum lipids, uncontrolled lipolysis, and dysregulation of adipogenesis and lipogenesis are involved in development of atherosclerosis and cardiovascular diseases. Atherogenic dyslipidemia is caused by different metabolic abnormalities including; increased cholesterol synthesis; increased production of triglyceride-rich lipoproteins, and increased high density cholesterol catabolism. It is believed that among these abnormalities, the pivotal role is played by increased hepatic production of lipoproteins (Arca *et al.*, 2012). Triglycerides are provided from *de novo* synthesized or extra hepatic fatty acids. Adipose tissue-derived free fatty acid is the largest extra hepatic source of fatty acid for triglyceride

synthesis (Arca *et al.*, 2012). In diabetes, deficiency of insulin in conjunction with glucagon or catecholamine-stimulated lipolysis increases fatty acid delivery to liver which may lead to ketoacidosis which is a life-threatening condition (Perilli *et al.*, 2013).

2.6.6 Amino Acid Metabolism

Metabolism of amino acids is believed to play a key role early in the development of type 2 diabetes. The risk of future diabetes would be at least four times higher in normoglycemic individuals with high fasting plasma concentrations of three amino acids namely; isoleucine, phenylalanine, and tyrosine, since concentrations of these amino acids were elevated up to 12 years prior to the onset of diabetes (Wang *et al.*, 2011).

2.7 Signs and Symptoms of Diabetes Mellitus

Type 1 diabetes develops gradually, but the symptoms may seem to come on suddenly. Some of the signs and symptoms include: being very thirsty, nausea and vomiting, blurry eyesight, unexpected weight loss, sudden frequent urination in children especially at night, deep rapid breathing, dry skin and mouth, flushed face, fruity breath odor, nausea or vomiting; inability to keep down fluids, stomach pain etc. (Marshall *et al.*, 2009). Common symptoms of type 2 diabetes include: Polydipsia, polyuria, nocturia, lethargy, fatigue, polyphagia, tiredness, weight loss, blurring of vision, pruritus vulvae, balanitis, nausea; headache, hyperphagia; predilection for sweet foods, mood change, irritability, difficulty in concentrating, and apathy (ADA, 2014).

2.8 Methods and Criteria for Diagnosing Diabetes Mellitus

Several methods for diagnosis of diabetes mellitus are currently practiced. They are given below as published by the World Health Organization in 2006 in its report titled:

“definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia”. In the year 2011, the World Health Organization also accepted the use of HbA1c testing in diagnosing diabetes in its report titled: "use of glycosylated hemoglobin in the diagnosis of diabetes mellitus". These criteria are summarized below:

1. Presence of Diabetes symptoms (e.g. polyuria, polydipsia and unexplained weight loss for Type 1) plus:
    I. A random venous plasma glucose concentration of greater or equal to 11.1 mmol/L or
    II. A fasting plasma glucose concentration of greater or equal to 7.0 mmol/L (whole blood greater or equal to 6.1 mmol/l) or,
    III. Two hour plasma glucose concentration of greater or equal to 11.1 mmol/L two hours after 75g anhydrous glucose in an oral glucose tolerance test (OGTT).
2. With no symptoms, diagnosis should not be based on a single glucose determination but requires confirmatory plasma venous determination. At least one additional glucose test result on another day with a value in the diabetic range is essential, either fasting, from a random sample or from the two hour post glucose load. If the fasting random values are not diagnostic the two hour value should be used.
3. The criteria for diagnosing gestational diabetes are different. Gestational diabetes should be diagnosed if the woman has either:
    I. a fasting plasma glucose level of 5.6mmol/L or above or
    II. a 2-hour plasma glucose level of 7.8mmol/L or above.

Glycated hemoglobin (HbA1c) of 48mmol/L (6.5%) is recommended as the cut off point for diagnosing diabetes mellitus. A value of less than 48mmol/L (6.5%) does not exclude diabetes diagnosed using glucose tests. In patients without symptoms of diabetes the laboratory venous HbA1c should be repeated. If the second sample is less than 48mmol/L (6.5%) the person should be treated as at high risk of diabetes and the test should be repeated in 6 months or sooner if symptoms develop (WHO, 2011).

2.9 Complications of Diabetes Mellitus

Diabetes is a group of chronic diseases characterized by hyperglycemia. Modern medical care uses a vast array of lifestyle and pharmaceutical interventions which are aimed at preventing and controlling hyperglycemia. In addition to ensuring the adequate delivery of glucose to the tissues of the body, treatment of diabetes also attempts to decrease the likelihood that the tissues of the body are not injured by the effects of hyperglycemic conditions. The importance of protecting the body from hyperglycemia is very critical, since direct and indirect effects on the human vascular tree are the major source of morbidity and mortality in both type 1 and type 2 diabetes mellits (Fowler, 2011). Generally, the injurious effects of hyperglycemia are separated into microvascular complications (diabetic nephropathy, neuropathy, and retinopathy) and macrovascular complications (coronary artery disease, peripheral arterial disease, and stroke) (Fowler, 2011) as it is partly depicted in Figure 2.4 below. Diabetes predisposes patients to opportunistic infections, vascular and neural pathologies. Based on its pathophysiology, diabetes mellitus can be acute or chronic.

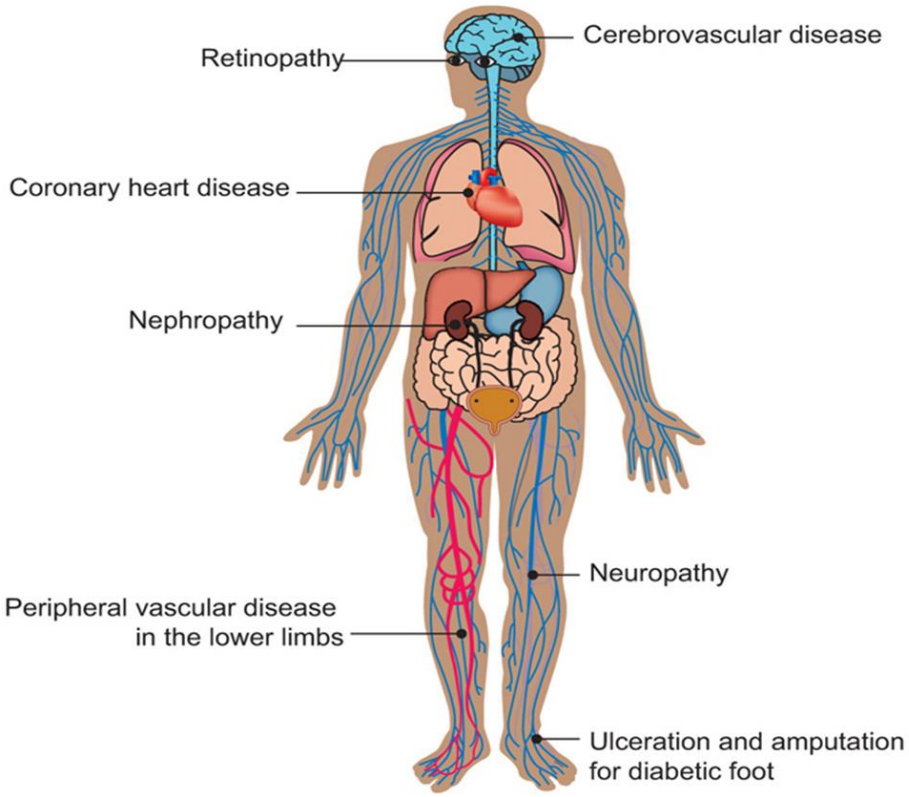

Figure 2.4: Microvascular and Macrovascular complications of Diabetes

(Source-WHO, 2016)

2.9.1 Acute Complications of Diabetic Mellitus

Acute complications of diabetes mellitus include diabetic ketoacidosis (DKA) and non-ketotic hyper-osmolar state (NKHS). Diabetic ketoacidosis is seen primarily in individuals with type 1 diabetes mellitus while nonketotic hyperosmolar state is prevalent in individuals with type 2 diabetes mellitus. The two disorders are associated with absolute or relative insulin deficiency, volume depletion and altered mental state (Kitabchi *et al*., 2008). In DKA, insulin deficiency is combined with counter regulatory hormone excess with respect to glucagon, catecholamines, cortisol and growth hormone.

The decreased ratio of insulin to glucagon promotes gluconeogenesis, glycogenolysis and ketone body formation in the liver and also increases free fatty acid and amino-acid delivery from fat and muscle to the liver (Eugen-Olsen *et al*., 2010). Ketosis results from a marked increase in free fatty acid release from adipocytes due to increased lipolysis. In DKA, nausea and vomiting are often present. Severe DKA may result in lethargy and central nervous system depression eventually leading into coma. Cerebral edema, an extremely serious complication, is seen most frequently in children. Non-ketotic hyper-osmolar state is most commonly seen in elderly individuals with type 2 diabetes mellitus. Its most prominent features include polyuria, postural hypotension, and a variety of neurological symptoms including altered mental state, lethargy, seizure and possibly coma. Insulin deficiency and inadequate fluid intake are the underlying causes of NKHS (Kitabchi *et al*., 2008). Hyperglycemia consequent to insulin deficiency induces an osmotic diuresis leading to excessive intravascular volume depletion (Kitabchi *et al*., 2008).

2.9.2 Chronic Complications of Diabetes Mellitus

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

cerebral disorders (Tripathi & Srivastava, 2006). Early in the course of diabetes, intracellular hyperglycemia causes abnormalities in blood flow and increased vascular permeability. This reflects decreased activity of vasodilators such as nitric oxide, increased activity of vasoconstrictors such as angiotensin II and endothelin-1 and production of permeability factors such as vascular endothelial growth factor. In diabetes, arterial endothelial dysfunction seems to involve both insulin resistance specific to the phosphotidylinositol–3-OH kinase pathway and hyperglycemia (Oechslin *et al*., 2000).

(a) Diabetic Retinopathy

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

(b) Diabetic Neuropathy

The precise nature of injury to the peripheral nerves from hyperglycemia is not known but is likely related to mechanisms such as polyol accumulation, injury from advanced glycosylated end products and oxidative stress. Peripheral neuropathy in diabetes may manifest in several different forms, including sensory, focal/multifocal and autonomic neuropathies (Vincent *et al*., 2011). About half of all people with diabetes have some degree of neuropathy, which can be polyneuropathy, mono-neuropathy and/or autonomic neuropathy (Tapp *et al*., 2003). Polyneuropathy is the most common form of neuropathy in diabetes (Tripathi & Srivastava, 2006).

There is loss of peripheral sensation which, when coupled with impaired microvascular and macrovascular junction in the periphery, can contribute to non-healing ulcers, the leading cause of non-traumatic amputation. There is thickening of axons, decrease in microfilaments, and capillary narrowing involving small myelinated or non-myelinated C-fibers. It can occur both from direct hyperglycemia-induced damage to the nerve parenchyma and from neuronal ischemia leading to abnormalities of microvessels, such as endothelial cell activation, pericyte degeneration, basement membrane thickening and monocyte adhesion (Sorensen *et al*., 2006). Pure sensory neuropathy is relatively rare and associated with periods of poor glucose control or considerable fluctuation in diabetes control. It is characterized by isolated sensory findings without signs of motor neuropathy. Symptoms are typically most prominent at night. Mononeuropathy is less common than polyneuropathy and includes dysfunction of isolated cranial or peripheral nerves. Autonomic neuropathy can involve multiple systems, including cardiovascular,

gastrointestinal, genitourinary, sudomotor and metabolic systems (Yagihashi *et al*., 2007).

(c) Diabetic Nephropathy

Diabetic nephropathy is defined by proteinuria greater than 500 mg in 24 hours, but this is preceded by lower degrees of proteinuria, or "microalbuminuria." Microalbuminuria is defined as albumin excretion of 30–299 mg per 24 hours (Fowler, 2008). Without intervention, diabetic patients with microalbuminuria typically progress to proteinuria with decreased glomerular filtration rate and end stage renal failure (Giorgino *et al*., 2004). This progression occurs in both type 1 and type 2 diabetes. Dysfunction of the glomerular filtration is attributed to changes in synthesis and catabolism of various glomerular basement membrane macromolecules such as collagen and proteoglycans, leading to an increase in glomerular basement thickening (Chaturvedi *et al*., 2001). Another possible mechanism to explain the increase in permeability of the glomerulus is the increase in renal vascular endothelial growth factor (VEGF) levels observed in preclinical models of diabetes, since VEGF is both an angiogenic and a permeability factor (Oechslin *et al*., 2000).

(d) Cardiovascular Disorders

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

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

(e) Diabetic Hypertension

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

atherosclerosis, and is also associated with an increased mortality from strokes and heart attacks (Bastaki, 2005).

(f) Infections during Diabetic Condition

Individuals with diabetes mellitus exhibit a greater frequency and severity of infection (Bertoni *et al*., 2001). The reasons for this include incompletely defined abnormalities in cell-mediated immunity and phagocyte function associated with hyperglycemia as well as diminished vascularization secondary to long-standing diabetes (Mazade & Edwards, 2001). Many common infections are more frequent and severe in the diabetic population, whereas several rare infections are seen almost exclusively in the diabetic population. These include rhinocerebral mucormycosis and malignant otitis externa, which is usually secondary to *Pseudomonas aeruginosa* infection in the soft tissue surrounding the external auditory canal (Casqueiro *et al*., 2012). Pneumonia, urinary tract infection, and skin and soft tissue infections are all more common in the diabetic patients. The most common organisms causing infections in diabetic patients are *Staphylococcus aureus, Escherichia coli, Streptococcus pneumoniae* and *Mycobacterium tuberculosis* (Laupland *et al*., 2004). Diabetic patients have an increased rate of colonization of *S. aureus* in skin folds and nares and also have a greater risk of postoperative wound infections (Tripathi & Srivastava, 2006).

2.10 Strategies for Prevention of Diabetes Mellitus

The impacts of diabetes on individuals' health and its economic burden to society have made its prevention a major goal of the current era. In the past decade, major advances have been made in our understanding of the prevention of type 2 diabetes. Interventions

that can reverse impaired glucose regulation early in its course may be the key to primary prevention of the long-term complications of diabetes. Type 2 diabetes is a heterogeneous disorder characterized by two interrelated metabolic defects: insulin resistance coupled with impaired insulin secretion by β-cells in the pancreas (Kahn, 2003). Therefore, strategies that target these two mechanisms of improving insulin sensitivity and protecting β-cell function have become the focus of prevention efforts.

2.10.1 Weight Management

The prevalence of type 2 diabetes is said to be 3 to 7 times higher in obese than in normal-weight adults, and those with a BMI over 35 kg/m^2 are 20 times more likely to develop diabetes than those with a BMI between 18.5 and 24.9 kg/m^2 (Pi-Sunyer, 2009). In addition, weight gain during adulthood is also directly correlated with an increased risk of type 2 diabetes. Obesity also complicates the management of type 2 diabetes by increasing insulin resistance and blood glucose concentrations.

Obesity is an independent risk factor for dyslipidemia, hypertension, and cardiovascular disease (ADA, 2015), thus increases the risk of cardiovascular complications and cardiovascular mortality in patients with type 2 diabetes. Weight loss is an important goal for overweight or obese persons, particularly those with type 2 diabetes, because it improves glucose control. Moreover, improvement in fasting blood glucose is directly related to the relative amount of weight lost. Weight loss has important additional health benefits in patients with diabetes because it improves other risk factors for cardiovascular disease (Inzucchi *et al*., 2012).

Weight loss protects against Type 2 diabetes but it is hard to maintain with behavioral modification alone. Bariatric surgery as a means of achieving weight loss has proven to

be successful in diabetes prevention. Patients with severe obesity have the highest risk of type 2 diabetes and where bariatric surgery is currently the only treatment that typically results in large, sustained weight losses. The results of an analysis done by Keating *et al*., (2015) has showed that bariatric surgery, as compared with usual care, reduces the long-term incidence of type 2 diabetes by 78% in obese patients. Among patients with impaired fasting glucose, bariatric surgery reduced the risk by 87%, and type 2 diabetes did not develop in approximately 10 of 13 obese patients who underwent bariatric surgery. It has been shown that bariatric surgery results in long-term weight loss and reduces the incidence of several hard end points (Sjöström *et al*., 2004).

2.10.2 Proper Nutrition

The importance of preventing type diabetes is highlighted by the substantial worldwide increase in the prevalence of diabetes in recent years. Genetic susceptibility appears to play a powerful role in the occurrence of diabetes. However, given that population gene pools shift very slowly over time, the current epidemic of diabetes likely reflects changes in lifestyle leading to diabetes. Lifestyles changes characterized by increased energy intake and decreased physical activity appear to have together promoted overweight and obesity, which are strong risk factors for diabetes (ADA, 2008).

Both the Finnish Diabetes Prevention study and the Diabetes Prevention Program focused on reduced intake of calories (using reduced dietary fat as a dietary intervention). Of note, reduced intake of fat, particularly saturated fat, may reduce risk for diabetes by producing an energy-independent improvement in insulin resistance, as well as by promoting weight loss. It is possible that reduction in other macronutrients (e.g.,

carbohydrates) would also be effective in prevention of diabetes through promotion of weight loss (Navarro *et al*., 2015).

Several studies have provided evidence for reduced risk of diabetes with increased intake of whole grains and dietary fiber. Whole grain–containing foods have been associated with improved insulin sensitivity, independent of body weight, and dietary fiber has been associated with improved insulin sensitivity and improved ability to secrete insulin adequately to overcome insulin resistance (Ley *et al*., 2014). Low–glycemic index foods that are rich in fiber and other important nutrients are to be encouraged. A 2010 American Diabetes Association statement reviewed this issue in depth, and issues related to the role of glycemic index and glycemic load in diabetes management are addressed in more detail in the carbohydrate section of this document (ADA, 2010).

Observational studies suggest a U- or J-shaped association between moderate consumption of alcohol (one to three drinks per day) and decreased risk of type 2 diabetes mellitus, coronary heart disease (CHD), and stroke. However, heavy consumption of alcohol (greater than three drinks per day), may be associated with increased incidence of diabetes (Athyros *et al*., 2008). There are also specific vitamins such as D, E, C and K and minerals e.g. magnesium that have been associated with lower incidences of diabetes. The importance of proper nutrition observation can lead to the improvement in glucose control, weight management, quality life and reducing cardiovascular risk, severe hypoglycemia, diabetic distress and diabetic ketoacidosis (Smart *et al*., 2009).

2.10.3 Physical Activity

Sedentary lifestyle is one of the most important risk factors for T2DM. Physical activity is an important component of any weight management program. Most patients with type 2 diabetes mellitus can benefit from increased activity. Aerobic exercise improves insulin sensitivity and may improve glycaemia markedly in some patients. A previously sedentary patient should start activities slowly (Balducci *et al*., 2010). Older patients, patients with long-standing disease, patients with multiple risk factors, and patients with previous evidence of atherosclerotic disease should have a cardiovascular evaluation, probably including an imaging study, prior to beginning a significant exercise regimen. A study by (Balducci *et al*., 2010) has showed that a supervised, facility-based exercise training program, when added to standard treatments for type 2 diabetes mellitus, yields better results than does simply counseling patients to exercise.

Another study (Loimaala *et al*., 2009) found that a long-term endurance and strength training resulted in improved metabolic control of diabetes mellitus and significant cardiovascular risk reduction, compared with standard treatment. The mechanisms by which exercise produces positive results in patients with diabetes include improvement in insulin sensitivity and glucose disposal in the skeletal muscle, expression of nitric oxide synthase in the endothelial cells, improvement in obesity, and body fitness (Loimaala *et al*., 2009).

2.10.4 Pharmacological Therapy

Drugs from several classes have been studied for the prevention of diabetes. However, most medical regulatory authorities including FDA have not approved any drug for the

treatment of prediabetes or the prevention of type 2 diabetes so far (Ibarrola-Jurado *et al*., 2012). In summary, the drugs are;

Metformin: - The ADA recommends that, in addition to lifestyle counseling, metformin should be considered in selected patients with prediabetes (ADA, 2010). In a study, metformin of 1700 milligrams daily was found to be about half as effective as lifestyle intervention in reducing diabetes risk among subjects with elevated fasting and post-load plasma glucose concentrations (Avanzini *et al*., 2011).

Thiazolidinediones:- Analysis of available data from a Diabetes Prevention Program (DPP) study suggests that troglitazone was effective in preventing diabetes. In a Diabetes Reduction Assessment with Ramipril and Rosiglitazone Medication trial, investigators concluded that rosiglitazone at 8 milligrams daily reduces the incidence of type 2 diabetes mellitus in patients with IFG/IGT (DeFronzo *et al*., 2011).

Acarbose: - Acarbose of 100 milligrams three times a day has been shown in a study to prevent non-insulin dependent diabetes mellitus and to reduce diabetes rates by approximately 25% in patients at high risk for the development of type 2 diabetes according to Dream Trial Investigation report of 2006.

2.11 Strategies for Treatment of Diabetes Mellitus

The goals in caring for patients with diabetes mellitus are to eliminate symptoms and to prevent or at least slow the development of diabetic complications. Micro-vascular (i.e. eye and kidney disease) risk reduction is accomplished through control of glycaemia and blood pressure while macro-vascular (i.e. coronary, cerebrovascular, peripheral vascular) risk reduction, is achieved through control of lipids and hypertension, smoking cessation

while metabolic and neurologic risk reduction is achieved through control of glycaemia (WHO, 1994).

2.11.1 Nutrition Therapy

Medical nutrition therapy is an important component of healthy lifestyle which remains a cornerstone of diabetes prevention and management. Medical nutrition therapy has been shown to accrue sustained reduction in hemoglobin A1C in diabetic patients (Funnell *et al*., 2009) and also in the improvement in lipid profile and blood pressure in non-diabetic individuals (Van Horn *et al*., 2008). Several studies have shown that dietary measures are effective in weight reduction irrespective of the composition, provided there is adequate energy restriction, reduction in saturated fat, and adequate provision of dietary fiber (Stern *et al*., 2004). Lower consumption of total and saturated fat and processed foods, and higher consumption of fibers, whole grains, fruits, and vegetables have been shown to improve glucose control in patients with diabetes (Brennan *et al*., 2010).

2.11.2 Bariatric Surgery

In morbidly obese patients, bariatric surgery has been shown to improve diabetes control and, in some instances to normalize glucose tolerance. In 2011, the International Diabetes Federation Task force on Epidemiology and Prevention of Diabetes released a position statement on bariatric surgery. The task force recommended bariatric surgery as an appropriate treatment for people with type 2 diabetes mellitus and obesity who have been unable to achieve recommended treatment targets using medical therapies, particularly if other major comorbidities exist (Dixon *et al*., 2011). In 2013 Kashyap and colleagues

demonstrated that bariatric surgery can improve glucose control in patients with type 2 diabetes (Kashyap *et al*., 2013).

2.11.3 Conventional Anti-Diabetic Drugs

Diabetes is a chronic medical condition, which means that even though it can be controlled, it will last a lifetime. The choice of antihyperglycemic agents in diabetes mellitus is guided by medical needs of the patient and treatment goals, potency of the agent in achieving optimum glucose control, tolerability and side effect profile, ease of administration and convenience, cost effectiveness, and other beneficial extra glycemic effects (ADA, 2013).

There are different approaches to the treatment of diabetes. Generally, current therapeutic strategies for type 2 diabetes mellitus are limited and involve insulin and four main classes of oral antidiabetic agents that stimulate pancreatic insulin secretion, reduce hepatic glucose production, delay digestion and absorption of intestinal carbohydrate or improve insulin action (Gough & Narendran, 2010). The hyperglycemia observed in diabetes mellitus is the result of a mismatch between the quantity of insulin necessary to regulate metabolic processes and the amount of insulin being secreted by the β-cells. Insulin replacement therapy is the mainstay for patients with type 1 diabetes mellitus while diet and lifestyle modifications are the basis for the treatment and management of type 2 diabetes mellitus in its initial stages (Powers & D'Alessio, 2011).

Insulin is also important in type 2 diabetes mellitus when blood glucose levels cannot be controlled by diet, weight loss, exercise and oral medications (Gough & Narendran, 2010). Insulin is the most potent glucose-lowering agent, with hypoglycemia being the only major dose-limiting factor. Insulin has progressively more side effects as the dose is

increased and may be administered intravenously or intramuscularly. However for long-term treatment, subcutaneous route is preferred (Bastaki, 2005). Insulin significantly reduces glucose concentrations by suppressing hepatic glucose production, increasing postprandial glucose utilization and improving the abnormal lipoprotein that is characteristic of insulin resistance. Insulin therapy may also decrease or eliminate the effects of glucose toxicity by reducing hyperglycemia to improve insulin sensitivity and β-cell secretory function (Søeborg *et al*., 2009).

Oral hypoglycemic agents are important in the treatment of type 2 diabetes mellitus where there are residual functioning pancreatic β-cells. Oral hypoglycemic agents include sulphonylureas, biguanides, α-glucosidase inhibitors, thiazolidinediones and more recently meglitinide analogues (Bailey, 2009). Sulphonylureas act directly on the islet β-cells to close ATP-sensitive K+ channels, which stimulate insulin secretion. The efficacy of sulphonylureas depends on the presence of a functional pancreas. The first generation of sulfonylureas included tolbutamide, acetohexamide, tolazamide, and chlorpropamide. A second generation of more effective sulphonylureas has been developed and includes glibenclamide, glipizide, gliclazide, and glimepiride (Tripathi & Srivastava, 2006).

Metformin is the most commonly used biguanide. Its mechanisim of action is not fully understood although it is antihyperglycemic and does not induce hypoglycemia. When used alone or in combination with a sulfonylurea, metformin improves glucose control and lipid concentrations in patients who are nonresponsive to sulphonylureas (Bailey, 2008**)**. Metformin improves insulin resistance in the liver, skeletal muscle and adipose tissue. It also reduces hepatic glucose output. Its efficacy in reducing fasting plasma

glucose and postprandial glucose concentrations is similar to that of sulphonylureas but in contrast it does not cause weight gain or hypoglycaemia (Bastaki, 2005).

Rosiglitazone and pioglitazone are the two thiazolidinediones currently in use. Thiazolidinediones act by lowering insulin resistance in peripheral tissue, but an effect to lower glucose production by the liver has also been reported (Semple *et al*., 2006). Thiazolidinediones increase glucose transport into muscle and adipose tissue by enhancing the synthesis and translocation of specific forms of the glucose transporter proteins. The thiazolidinediones can also activate genes that regulate free fatty acid metabolism in peripheral tissue, thus lowering triglycerides and non-esterified fatty acid levels and inducing differentiation of adipocytes (Tripathi & Srivastava, 2006). They are used effectively in combination with other classes of antidiabetic agents (Tripathi & Srivastava, 2006).

The meglitinide analogues are a newer class of drugs developed from the meglitinide portion of sulphonylureas. They work by improving early-phase insulin secretion and examples are repaglinide and nateglinide (Blickle, 2006). Repaglinide is derived from the nonsulphonylurea moiety of glibenclamide whereas nateglinide is derived from the amino acid D-phenylalanine. The meglitinides are rapid-acting insulin secretagogues that have a fast onset and short duration of action resulting in more physiological secretion of insulin from the β-cell without causing continued elevation of insulin in the post-absorptive phase, thus reducing glycaemia without increasing the risk of hypoglycemia.

Alpha-glucosidase inhibitors competitively inhibit α-glucosidases that are associated with the brush border membrane of the small intestine and are responsible for the digestion of complex polysaccharides and sucrose. This slows carbohydrates digestion and lowers

post-prandial hyperglycemia. They can be used as monotherapy or in combination with other oral antidiabetic drugs (Rabasa-Lhoret & Chiasson, 2003). The three α-glucosidase inhibitors that have been developed are acarbose, miglitol, and voglibose (Bailey & Day, 2009).

2.12 Herbal Remedies

Medicinal plants form an integral component of ethno-medicine for both human and animal uses all over the world. They have been in use for centuries to treat illness and improve health, and still account for approximately 80% of medical treatments in the developing world (WHO, 2008). By use of a few cases, one can easily appreciate the long history of ethno-medicine which has continued to have an impact on today's field of medicine. The use of herbal remedies has gained a lot of attention due to its perceived benefits such as safety, affordability and client satisfaction which can generally be classified as therapeutic and economic benefits (Iwu *et al*., 1999). Herbs are generally defined as any form of plant or plant product, including leaves, stems, flowers, roots and seeds (Bent, 2008). Herbal products may contain a single herb or a combination of several different herbs believed to have complementary effects.

According to WHO definition, there are three forms of herbal medicines namely; raw plant materials, processed plant materials and medicinal products that contain active ingredients such as aerial or underground plants material or a combination thereof, whether in crude state or as plant preparations. The reliance on medicinal plant products is not confined to Africa or the developing world, but it also exists in the developed nations (Smith-Hall *et al.* 2012). However their widespread use in developing countries

may be attributed to poverty which makes the conventional life-saving drugs unaffordable and inaccessible due to financial constraints (Nyazema, 1986).

The use of herbal medicine has thus gained popularity in primary health care of the poor in developing countries and even in countries where conventional medicine is predominant in national health care system (WHO, 1997). Majority of the population in developing countries therefore rely on medicinal plants to meet health care needs, and this has been going on even in situations where conventional medicine is available due to historical and cultural reasons (WHO, 1999). In Kenya, nearly 80% of people live within 5 kilometers of a health facility but medical services are not always available as health facilities often lack basic drugs, basic services and amenities and the cost of modern medicine is high (NCAPD, 2008). In addition, there is still shortage of health professionals and the ratio of doctors to the population remains low at 15 per 100,000 (NCAPD, 2005). These factors result in promoting the usage of herbal remedies in Kenya by majority of the communities.

Some pharmaceutical companies are investing in research on how to use these traditional remedies as ‘lead’ compounds for new drugs. For example, it has been noted that out of the newly approved drugs reported between 1983 and 1994, drugs of herbal origin predominated (78 %) in the antibacterial area, while 61 % of the 31 anticancer drugs approved in the same period were either natural products, nature-derived products or compounds modeled on natural product parents. Furthermore, 50 % of the best-selling pharmaceuticals in 1991 were either natural products or their derivatives (Gupta & Amartya, 2012). Advances in pharmaceutical technology have led to investigation and ultimate isolation of pure active compounds from crude drugs which are now produced at

commercial levels. The most common examples of modern medicines derived from natural lead compounds include anticancer agents like vincristine and vinblastine from *Catharanthus roseus,* antimalarial quinine from *Cinchona spp* and the anticholinergic agent atropine from *Atropa belladona* and *Atropa acuminata* as well as opioid analgesic morphine (Gupta & Amartya, 2012). Herbal remedies reputed to possess antidiabetic activity may also be investigated with a view to developing potent and efficacious antidiabetic agents.

2.12.1 Alternative Medicine for Diabetes Mellitus

The field of herbal medicines research has been gaining notable importance in the last few decades and the demand for the use of natural products in the treatment of diabetes is increasing worldwide. Presently, over 800 traditional plant based treatments for diabetes have been reported, although only a small number of them have received scientific and medical evaluation to assess their efficacy (Kumar *et al.*, 2018). The best example is metformin which is an effective oral glucose-lowering agent which was developed based on the use of *Galega officinalis* to treat diabetes that is rich in guanidine, the hypoglycemic component (Kumar *et al.*, 2018).

Most research work on diabetic drugs is targeted on plants and plant-derived products. Many natural products and their analogues have been identified as potent antidiabetic agents. Many of them have been scientifically explored for their usefulness in managing diabetes with reports having been acknowledged and published in a number of scientific journals. For instance some Kenyan plants like *Bidens pilosa, Erythrina abyssinica, Aspilia pluriseta, Strychnos henningsii* and *Catha edulis* have been shown to significantly

lower blood glucose to normal and as effectively as insulin and at times beyond the lowering effect of insulin in alloxan-induced diabetic mice (Piero *et al*., 2012).

There are several mechanisms through which herbs act to control the glucose level. They have more or less similar actions to the synthetic drugs for instance; stimulation of insulin secretion*;* free radical scavenging activity*;* increase metabolism of glucose*;* lowers plasma glucose level; reduction of intestinal absorption of glucose*;* reduce blood glucose and glycosylated hemoglobin*;* increase glucose uptake*;* increase glycogenesis, decrease glycogenolysis and gluconeogenesis, and beta cell rejuvenation, regeneration and stimulation (Mishra *et al*., 2011).

Plants contain several phytochemicals, including alkaloids, flavonoids, glycosides, glycolipid, galactomannan, polysaccharides, peptidoglycan, hypoglycans, guanidine, steroids, carbohydrates, glycopeptides, terpenoids, amino acids, saponins, dietary fibres and inorganic ions that affect various metabolic cascades, which directly or indirectly affect the level of glucose in the human body (Mngeni, 2017). These phytochemicals possess potent hypoglycemic, anti-hyperglycemic and glucose suppressive activities (Saxena *et al*., 2006). These anti-diabetic effects are achieved by either increase in serum insulin level or increase in the production of insulin from pancreatic β-cells; inhibit glucose absorption in the gut; stimulate glycogenesis in liver or increase glucose utilization by the body (Gupta *et al*., 2008). These phytochemicals are also said to exhibit antioxidant, hypolipidemic, anti-cataract activities, restored enzymatic functions, repaired and regenerated pancreatic islets and alleviated liver and renal damage (Mukherjee *et al*., 2006).

The World Health Organization Expert Committee on diabetes recommended that traditional medicinal herbs need be explored further (Bailey & Day, 1989). The major obstacle in the integration of herbal medicine into modern medical practices has been the lack of scientific and clinical data proving their efficacy and safety. Therefore, there is need for clinical research in herbal drugs by using appropriate bioassays for biological standardization, pharmacological and toxicological evaluation, and to develop various animal models for toxicity and safety evaluation. It is also important to establish the active component(s) from these plant extracts (Kumar *et al*., 2018). A scientific investigation of traditional herbal remedies for diabetes may provide valuable paths for the development of alternative drugs and strategies. Alternatives are clearly needed for better management of diabetes because of high cost and poor availability of current therapies for many poor rural populations particularly in developing countries.

In spite of the presence of known antidiabetic medicines in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease (Bhattaram *et al.*, 2002). Many traditional plant treatments for diabetes are used throughout the world. Plant drugs (Bailey & Day, 1989) and herbal formulations (Annapurna *et al.*, 2001) are frequently considered to be less toxic and have fewer side effects compared to synthetic ones. Based on the WHO recommendations, hypoglycemic agents of plant origin used in traditional medicine are important (WHO Expert Committee on Diabetes Mellitus, 1980). Roots of *Maerua decumbens* have been for instance reported as being used for the treatment of diabetes by communities of Elgeyo-Marakwet County of Kenya (Kigen *et al.*, 2014). However, its antidiabetic efficacy and safety has not been evaluated.

2.12.2 Alternative Medicinal Uses of *Maerua decumbens*

Maerua decumbens belongs to Capparaceae family and genus Maerua. There is little literature records concerning this plant. *Maerua decumbens* is fairly widespread in East and Southern Africa. *Maerua decumbens* occurs in hot dry areas, in deciduous woodland, bush land and thickets, as well as grasslands. Some of the known local names for this plant by Kenya communities include;- Chepyetabei (Kigen *et al*, 2014), Hagarniama (Borana), Munatha (Kamba), Ketit Ap Teita (Kipsigis), Amoyo (Luo), Olkemet-me (Masai), Kukube-tari (Oromo), Chepluswo (Pokot), Lamuyeg (Samburu), Abar Moq, Ohia sagara (Somali), Monongwe, Yubuluswa (Tugen), Lamayoki (Njemps), Eerut (Turkana) (Beentje, 1994). Other local names from other Kenyan communities include chebiliswo, abiro, dawa-nyoka, gindarithi, haluf, olkiage, mkulube, lamaloki, abarmog, dawa-aaze, bariyub, mutunguarithi, kukube-dik dik, chebillio, chepiliowo, mundarith, lamayokin, kangalige, and agarnyaab (Quattrocchi, 2012). *Maerua decumbens* grows perennially and it is a small shrub growing up to 3 meters high, with many stems arising from a tuberous rootstock (Thulin, 2006). The leaves are greyish green and broadly ovate (Figure 2.5). The flowers are white; the fruits are ellipsoid in shape, and borne on a long stalk. The ripe fruits are yellow or orange.

Figure 2.5: Uprooted *Maerua decumbens* Plant (Thulin, 2006)

It mostly occurs naturally in the arid and semi-arid areas in Kenya, and is used traditionally by rural communities for medicinal and water clarification purposes. There are published and unpublished reports on the use of this plant by various communities as an herbal remedy. The indigenous communities of Samburu and Baringo districts, Kenya use the roots to clarify and disinfect water (Kipkemboi, 2011). Historically, Tugen, pokot and Illchamus communities in Kenya use the raw or boiled roots of *M. decumbens* to treat stomachache and livestock intestinal worms; roots and bark are soaked in warm water and the liquid is drunk to treat venereal diseases; roots are used as purgative; roots are boiled and mixed with broth for health and vitality; Leaves and flowers are boiled in a little water and the mixture applied in a poultice and bandaged firmly on sore joints; leaves are used as fish poison; leaves juices are used to treat allergy; root bark is chewed by pregnant women because of its sweet taste (Quattrocchi, 2012).

The *Maerua decumbens* leaf extracts have also been established to exhibit antifungal activity and the phytochemical analysis revealed the presence of flavonoids and cardiac glycosides (Kiswii *et al.*, 2014). Roots of *Maerua decumbens* have been for instance reported as being used for the treatment of diabetes by communities of Elgeyo-Marakwet County of Kenya (Kigen *et al.*, 2014). However, this antidiabetic efficacy claim and safety has not been documented and therefore, it formed one of the basis for the current study.

2.13 Animal Models for Screening Diabetes Mellitus

Different animal models of type 1 and type 2 diabetes mellitus have been used for screening for antidiabetic activity of new drugs. These animals range from surgical models, genetic models, various animal strains that spontaneously develop diabetes, and chemical models of diabetes mellitus (King, 2012). The species of animal to be used for antidiabetic screening is determined by several factors. Generally, smaller animals are more manageable and cheaper hence, rats and mice are the most commonly used (King, 2012).

One of the most commonly used methods for inducing diabetes is by damaging the pancreas through administration of diabetic inducer chemicals such as streptozotocin (STZ) and alloxan. These animal models mimic several characteristics of the human disease (Islam, 2012). Chemically induced models of diabetes mellitus enable evaluation of blood glucose following treatment with a new test drug. The results are then compared to non-diabetic or diabetic animals treated with conventional antidiabetic drugs. A type 1 diabetic rat model has been developed using the Wistar rat by injecting adult rats with a single dose of streptozotocin at 45 mg/kg, intraperitoneally (Gayathri & Kannabiran,

2008). The streptozotocin-induced Wistar rat develops complications associated with hyperglycemia, similar to the human diabetic situation. Thus this diabetic rat is a suitable model for the investigations into the pathology of diabetes mellitus and complications related to the disease as well as possible interventions (Gayathri & Kannabiran, 2008). Rodents also tend to show a substantial gender difference in STZ sensitivity. Male mice and rats tend to be more susceptible to STZ-induced diabetes. This reduced sensitivity experienced by females may be attributed to oestradiol's ability to protect pancreatic β-cells from apoptosis induced by oxidative stress (Deeds *et al*., 2011).

Streptozotocin (2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose) (STZ) is a broad spectrum antibiotic synthesized by *Streptomycetes achromogenes.* It is used clinically for the treatment of metastatic islet cell carcinoma of the pancreas. Experimentally, it has been used in different animal species to induce both type 1 and type 2 diabetes mellitus (Lenzen, 2008). The frequently used single intravenous dose in adult rats to induce type 1 diabetes mellitus is 40-60 mg/kg body weight but higher doses are also used.

Streptozotocin activity in β-cells is characterized by alterations in blood insulin and glucose concentrations. Hyperglycemia and a drop in insulin are observed two hours after injection. This is followed six hours later by hypoglycemia with high levels of blood insulin. Finally, hyperglycemia develops and blood insulin levels decrease. These changes in blood glucose and insulin concentrations reflect abnormalities in β-cell function (Deeds *et al*., 2011). STZ impairs glucose oxidation and decreases insulin synthesis and secretion (Lenzen, 2008). STZ is taken up by the pancreatic β-cells via glucose transporter GLUT2. The main reason for the STZ-induced β-cell death is

alkylation of DNA. The alkylating activity of STZ is related to its nitrosourea moiety, especially at the O6 position of guanine. Since STZ is a nitric oxide (NO) donor and NO was found to bring about the destruction of pancreatic islet cells, it was proposed that this molecule contributes to STZ-induced DNA damage (Dickson *et al*., 2016). However, the results of several experiments provide the evidence that NO is not the only molecule responsible for the cytotoxic effect of STZ. STZ was found to generate reactive oxygen species (ROS), which also contribute to DNA fragmentation and evoke other deleterious changes in the cells.

The formation of superoxide anions results from both STZ action on mitochondria and increased activity of xanthine oxidase (Lenzen, 2008). It was demonstrated that STZ inhibits the Krebs cycle and substantially decreases oxygen consumption by mitochondria. These effects strongly limit mitochondrial ATP production and cause depletion of this nucleotide in β-cells. Restriction of mitochondrial ATP generation is partially mediated by NO. Augmented ATP dephosphorylation increases the supply of substrate for xanthine oxidase (β-cells possess high activity of this enzyme) and enhances the production of uric acid which is the final product of ATP degradation. Xanthine oxidase then catalyzes the reaction in which the superoxide anion is formed. As a result of superoxide anion generation, hydrogen peroxide and hydroxyl radicals are formed. STZ-induced DNA damage activates poly adenosine diphosphate ADP-ribosylation. This process leads to depletion of cellular Nicotinamide adenine dinucleotide (NAD), further reduction of the ATP content and subsequent inhibition of insulin synthesis and secretion (Lenzen, 2008).

Streptozotocin causes alkylation or breakage of DNA strands and a consequent increase in the activity of poly-ADP-ribose synthetase, an enzyme which depletes NAD in β-cells finally leading to energy deprivation and death of β-cells is reported (Lenzen, 2008). The potent alkylating properties of STZ are the main cause of its toxicity. However, the synergistic action of both NO and reactive oxygen species may also contribute to DNA fragmentation and other deleterious changes caused by STZ (Lenzen, 2008).

Alloxan has two distinct pathological effects: it selectively inhibits glucose-induced insulin secretion through specific inhibition of glucokinase, which is the glucose sensor of the beta cell, and it causes a state of insulin-dependent diabetes through its ability to induce ROS formation, which results in the selective necrosis of beta cells. These two effects can be assigned to the specific chemical properties of alloxan, the common denominator being selective cellular uptake and accumulation of alloxan by the beta cell (Lenzen, 2008).

2.14 Safety Concerns of Herbal Medicines

Toxicity is the degree to which a substance can harm humans or animals. It can be measured by its effects on the target organism, organ, tissue or cells. The toxic effects of a substance on animal physiology can range from minor changes such as reduced weight gain, small physiological alteration or change in the levels of circulating hormones, to severe effects in organ function leading to death. Intermediate levels of toxicity may cause pain and suffering (Hudson-Shore, 2016). Despite the growing market demand for herbal medicines, there are still concerns associated with not only their use, but also concerns on their safety. Less than 10% of herbal products in the world market are truly standardized to known active components and strict quality control measures are not

always diligently adhered to (Winston & Maimes, 2007). For majority of these products in use, little is known about their toxic composition.

Toxicological studies in the pharmaceutical field have been growing exponentially. These developments have been prompted by discovery of teratogenic effects of drugs such as thalidomide, exposure of chemicals to the environment and employees and by conduct and assessment of toxicity studies as part of good manufacturing practice (Traina, 1983). Some of the phytochemicals produced by plants against herbivorous and insects end up being harmful to humans, because of highly conserved biological similarities shared between both taxa as seen in most pathways involving protein, nucleic acid, carbohydrate and lipid metabolism as reported by (Kawashima *et al*., 2007).

Ecologically, a good number of alkaloids serve as feeding deterrents via agonistic activity on neurotransmitter systems (Wink, 2003). Similarly, some lipid soluble terpenes have been shown to have inhibitory properties against mammalian cholinesterase (Savelev *et al*., 2004). Saponins have been found to be potent surfactants which can disrupt lipid-rich cellular membranes of human erythrocytes and microorganisms which explain its potent antimicrobial properties of this group of phytochemicals (Francis *et al*., 2002). Another implication in the toxicity of certain herbs is the presence of toxic minerals and heavy metals like mercury, arsenic, lead and cadmium (Dwivedi & Dey, 2002). These facts support the need to have toxicity profiling of all herbal remedies.

Qualitative toxicity assessment of a chemical substance in laboratory animals gives information on its potential to cause toxic effects in humans or animals (Descote, 1996). To succeed in this exercise, an appropriate choice of control group must be selected, sufficient number of laboratory animals used and good selection of rigorous experimental

protocols. Furthermore, the severity of the effect described on major organs and the relevance of the mechanisms involved including the variations in different species assist in extrapolation of toxicological findings from laboratory animals to man (Descote, 1996). During the study, the target organ of toxicity in laboratory animals are identified, the mechanism of induced changes are noted and compared to the properties of the target site in man.

Determination of the toxic potential of new compounds constitutes a major part in drug development and it involves both *in vivo* and *in vitro* toxicological tests. These tests are very critical in the assessment of the safety of all pharmaceutical products before they are released for general use. Animal models are used in *in vivo* studies as indicators of human toxicity (Magna & Alan, 2007).

Toxicity testing on herbal extracts is carried out on the same principles as the conventional medicine. Scientific analysis of the effects of toxic chemical subtances can be performed either through *in vitro* (on cultured or mammalian cells) or *in vivo* (in living organism e.g. rat) methods. *In vivo* toxicity testing mainly employs the use of rats although other rodents may be used. Dogs and monkeys are restricted to advanced stages of testing (Amenya, 2011). Organization for Economic Cooperation and Development guidelines are used during acute and sub-acute oral toxicity testing (OECD, 1995). It is important to optimize the information obtained by using the smallest number of animals to comply with animal welfare regulations. Further, it is important to avoid excessive pain or tissue damage in the animals, pharmaceuticals with irritant or corrosive characteristics should not be administered in concentrations that produce severe toxicity after administration.

During toxicity studies, all the animals must be checked for morbidity, mortality and specific signs of toxicological relevance such as neurofunctional and neurobehavioral, opthalmological observation, body-weight and food/water intake. The key hematological parameters investigated are mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), hemoglobin levels, hematocrit levels, packed cell volume (PCV) total and differential leukocytes, erythrocytes and platelet counts. Clinical biochemistry is crucial to investigate major toxic effects on organs especially the kidney and the liver.

Some of the parameters include total protein, albumin, major electrolytes, total cholesterol, alanine aminotransferase, aspartate aminotransferase, creatinine and alkaline phosphatase that assist in hepatocellular evaluation. Pathological studies and gross necropsy are also done by examining the body, orifices, abdominal cavity, body weight and organ weight changes among others. In addition, histopathological studies are done on adrenals, lung, liver, kidney, testis, ovaries among others (OECD 407, 2008). These organs are considered to be the most important during toxicity studies in rodents and non-rodents (Michael *et al.* 2007).

Determination of weights of organs is necessary because organ to body weight ratios is commonly calculated and are considered more useful when body weights are affected (Michael *et al*. 2007). By carrying out toxicity tests, the effects of increase in dose on the mortality and other effects of the lethal dose that kills are determined. Estimating different levels of toxicity by use of LD_{50} for instance can help in estimating the probabilities of an outcome for a given individual in a population. The determination of

acute, sub-acute, sub-chronic and chronic toxic effects of the test compounds is therefore crucial (Traina, 1983).

2.14.1 Acute Toxicity Studies

Acute toxicity is caused by an agent when it is administered in one or more doses over a period not exceeding 24 hour and involves harmful effects to the organism through a single or short term exposure**.** Acute toxicity studies have also been used during the selection of starting doses for phase-I human studies and animal studies, and provide information relevant to acute overdosing in humans and animals (Klaassen & Watkins, 1996). The testing is based on the route of substance administration to the animal and therefore it is classified from Class-1 to Class-5 for oral, dermal, gas inhalation, vapor/dust/mist inhalation and injection. Dosing can be repeated during the administration of test material by a variety of routes of exposure, including gavaging which involves stomach intubation or forced feeding, injection, skin, painting and inhalation. The acute toxic class method, a step-wise procedure, involves the use of three animals of a single sex per step (OECD, 2001).

Depending on the mortality and/or moribund status of the animals, on average 2 to 4 steps may be necessary to allow judgment on the acute toxicity of the substance (Harizal *et al*., 2010). The OECD Guideline 423 of 2001 provides a reproducible method that uses few animals. According to this guideline, acute toxicity test measures relative toxicological response of an experimental organism to single or brief exposure to a test substance. The test organisms range from simple systems like brine shrimp to other animals like mice, rats, guinea pigs and rabbits. This test is also used to calculate median lethal dose (LD_{50}) of a substance, using various standardized acute toxic class method

(OECD, 2001). Following administration of a test product, animals are observed individually at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days in the case of delayed toxicities (OECD, 2001).

2.14.2 Sub-Acute Toxicity Studies

In this form of toxicity, adverse effects occur as a result of repeated daily dosing of a chemical or exposure to the chemical, for part of an organism's lifespan usually not exceeding 10 % of the animals' lifespan. Exposure for 28 days provides a first-hand indicator of potential sub -acute toxicity. The test is intended to investigate effects on a very broad variety of potential targets of toxicity. A repeat-dose study is performed to expose any deleterious changes in organ, hematological and biochemical indices that may arise in the course of repeated administration of a test substance and it usually ranges from weeks to a few months (Parasuraman, 2011). The duration of exposure is normally 28 days in rodents where results are used for hazard identification and risk assessment (OECD, 2008). All the knowledge gathered from the studies is used in selecting doses for repeat-dose studies as a source of preliminary identification of target organs of toxicity, and may also reveal delayed toxicity. Sub-acute toxicity studies in animals are essential for any pharmaceutical products especially those intended for human use.

Data that are generated include general parameters such as daily food consumption and water intake and body weight measurements. Other specific endpoints of toxicity assessed also include serum biochemical parameters, enzymatic and non-enzymatic liver oxidative stress indicators and hematological parameters. Various organs are also

examined for any gross pathological changes and tissue slices which are obtained from the respective organs are prepared for detailed histological examination.

Results of many sub chronic toxicity tests of various plant extracts have revealed that the major organs usually affected are the liver and kidneys. Hepatotoxic and nephrotoxic effects are also mostly expected, as the liver acts as the main detoxifying organ for chemical substances, while the kidney is the principal route of excretion for many chemical substances in their active or inactive forms (Abdulrahman *et al*., 2007). Liver injury associated with the use of herbal medicine ranges from mild elevation of liver enzymes to fulminant liver failure; and carcinogenesis (Maurer, 2008).

2.14.3 Sub-Chronic Toxicity Studies

Sub-chronic toxicity study involves is the ability of toxic substances to cause effects for more than one year but less than the lifetime of the exposed organism. This form of toxicity is studied for at least 90 days in animal models particularly rodents. The test is carried out after getting initial information on toxicity from acute or 28 day sub-acute toxicity study. This study provides information on likely hazards that may arise from repeated exposures over a prolonged period of time covering post-weaning, maturation and growth into adulthood (Yuet Ping *et al*., 2013). The study gives information on the major toxic effects; indicates target organs and the possibility of its accumulation. It can also provide an estimate of a Non-Observed-Adverse Effects Level of exposure which can be used in selecting dose levels for chronic studies and to establish safety criteria for human studies (OECD 408, 1998).

2.14.4 Chronic Toxicity Studies

Chronic toxicity is the ability of a substance or mixture of substances to cause harmful effects over an extended period, usually upon repeated or continuous exposure, sometimes lasting for the entire life of the exposed organism (Petrocelli, 1985). The three main routes for chronic study include oral, dermal and inhalation depending on the characteristic of the test substance and the predominant route of exposure in humans. The objectives of chronic toxicity studies include; identification of the hazardous properties of a chemical, identification of target organs, characteristic of dose-response relationship and identification of NOAEL or point of departure of Benchmark Dose. It also helps in identification of chronic toxic effects in human exposure levels and provision of data to test hypotheses regarding mode of action (OECD 452, 2008).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

Streptozotocin, Thiobarbituric Acid, and 1,1,3,3-Tetramethoxypropane were obtained from Sigma Aldrich, USA. Metformin was obtained from Glucophage®, Lipha Pharma Ltd., UK, and Glucometer (Wellion CALLA Light) was obtained from Med Trust, Germany. All other chemicals used were of analytical grade.

3.2 Ethical Approval

The animal protocols of this study were approved for ethical clearance by the Institutional Research Ethics Committee of the University of Eastern Africa, Baraton (Ref-REC: UEAB/9/3/2017) (Appendix IV). OECD principles and guidelines on the care and use of laboratory animals were followed during this study. The OECD guidelines followed were No 423 for testing of chemicals (Acute Oral Toxicty- Acute Toxic Class Method) and No 407 (Repeated Dose 28-day Oral Toxicty Study in Rodents).

3.3 Collection and Identification of Plant Material

The healthy roots of *Maerua decumbens* were freshly collected from around EMCEA Center, Elgeyo-Marakwet County, Kenya in January 2017. The medicinal plants were identified *in situ* by a local herbalist and placed in polyethene bags and transported and it was botanically identified by a taxonomist, Mr Denis Odhiambo of the Department of Biological Sciences, University of Eldoret, Kenya.

3.4 Preparation of Root Crude Extract

The roots of *M. decumbens* were washed to remove any debris, the dead bark peeled, chopped into small pieces, shade dried to constant weight and ground into homogenous powder using an electric mill. To obtain the extract, the root powder of *M. decumbens* were mixed (1:5 w/v) with methanol for 72 hours by cold maceration method with regular stirring. The mixture was filtered using Whatman No 1 filter paper. Then the combined extracts were concentrated under reduced pressure at 50^{o}C using Rotary Evaporator (AG CH-9230, Germany). The brick red syrup like crude extract was further dried in an oven at 40^{o}C for two days to remove remaining methanol. The percentage yield of the methanol extract was 15%. The concentrate was then put in an airtight container and stored at 4^{o}C until use in bioassay studies (Antia & Okokon, 2014). The suspension of methanolic extract was prepared by using normal saline before it was used for oral administration.

3.5 Qualitative Phytochemical Screening of the Root Extract

Phytochemical screening of the crude extract was carried out using standard phytochemical methods (Sofowora, 1993; Harbone, 1998; Evans, 2009) as detailed in subsequent sub-sections to reveal the presence or absence of the general classes of phytochemicals.

3.5.1 Test for Alkaloids

Mayer's reagent is an alkaloidal precipitating reagent used for the detection of alkaloids in natural products. Mayer’s reagent was freshly prepared by dissolving a mixture of mercuric chloride (1.36 g) and potassium chloride (5 g) in 100 ml of distilled water. Most

alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent to give a cream colored precipitate. About 1 g of extract was dissolved in 2 ml of 0.1 M hydrochloric acid and 2 drops of Mayer's reagent was added. A light yellow precipitate indicates the presence of alkaloids.

3.5.2 Test for Tannins

To the extract (0.5 g), 1% gelatin solution containing 10% sodium chloride was added. Formation of white precipitate indicated presence of tannins.

3.5.3 Test for Saponins

The extract (0.5 g) was shaken vigorously using 5 ml of distilled water in a test tube and warmed. The formation of stable foam indicates the presence of saponins.

3.5.4 Test for Flavonoids

The extract (0.5 g) was treated with 1 ml of 10% sodium hydroxide. A yellow solution that turns colorless on addition of 2 M hydrochloric acid indicates presence of flavonoids.

3.5.5 Test for Glycosides

About 0.5 g of extract was treated with 2 ml of glacial acetic acid which contained 2 drops of 10% ferric chloride solution. One ml of concentrated sulphuric acid was added along the sides of the test tube. A brown ring obtained at the interface indicates the presence of glycosides.

3.5.6 Test for Anthraquinones

Extract (0.5 g) was shaken with 10 ml benzene, filtered and 5 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of a pink, red or violet colour in the ammonia (lower) phase indicates the presence of anthraquinones.

3.5.7 Test for Terpenoids

To 0.5 g of the extract, 2 ml of chloroform was added and then it was evaporated to dryness. Two ml concentrated sulphuric acid was then carefully added and heated for two minutes. Greyish color indicates presence of terpenoids.

3.5.8 Test for Steroids

The extract (0.5 g) was dissolved in 2 ml of chloroform and then followed by 2 drops of ice cold concentrated sulphuric acid by the sides of the test tube. A red color in the lower chloroform layer indicates presence of steroids.

3.5.9 Test for Phenols

The extract (0.5 g) was treated with 5 ml of distilled water and then 3 drops of 5% ferric chloride solution was added. A dark green color formation indicates the presence of phenolic compounds.

3.6 Experimental Animals

Wistar albino rats (*Rattus norvegicus*) of 6-8 weeks old were obtained from Zoology Department, Chiromo Campus of the University of Nairobi, Kenya. The animals were housed in wire cages with sawdust and soft grass placed at the bottom of the cages. The animals were housed in cages in groups of 5 rats each per gender under standard laboratory conditions (Temperature, 25±2 ^{o}C, and 12 h light and 12 h dark cycle) in the animal house at the Department of Chemistry and Biochemistry, University of Eldoret. All animals were allowed free access to standard rodent chow (Unga Limited-Kenya) and drinking water *ad libitum.* The rats were acclimatized for a period of one week before initiation of the experiments. Their cages were cleaned of waste daily.

3.7 Acute Oral Toxicity Study of Root Extract in Rats

Six healthy, adult, female albino Wistar rats aged between 6-8 weeks and weighing between (150-195 g) were selected for this experiment. The acute toxicity study was carried out according to the limit test procedure of the OECD-423 guidelines with single dose exposure. A dose of extract at 2000 mg/kg b.wt was selected as the limit test dose and given orally (single dose in a 1 ml volume) to three female rats after an overnight fast. Specifically, after the rats were fasted, they were all weighed to obtain the baseline body weights before treatment started. The rats were grouped into two groups of 3 female rats each. The first group served as a normal control and they received vehicle (normal saline) at 1 ml/kg b.wt, while second group served as the test group and they received *M. decumbens* extract orally at dose of 2000 mg/kg b.wt. Food and water were provided *ad libitum* after extract administration. Animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 h (with special attention during the first 4 h), and daily thereafter for a period of 14 days. Once daily, cage-side visual observations included changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, autonomic (salivation, lacrimation and defecation) and central nervous system (drowsiness and tremors) changes. The fasting body weights of the animals were determined on days 0, 7 and 14. Signs or symptoms of treatment-related toxicity and/or mortality were monitored for up to 14 days (Algariri *et al*., 2014).

3.8 Sub-Acute Oral Toxicity Study of Root Extract in Rats

3.8.1 Experimental Design

This study was conducted in compliance with OECD guidelines No. 407 (OECD, 1994). The experimental animals were divided into four main groups of 10 rats each. Each group consisted of 10 6–8 week-old rats. Female rats weighing between 120-150 g, and 5 male rats weighing between 79-151 g per were placed in separate cages. The groups were treated daily with two oral doses of *M. decumbens* root extract (400 and 800 mg/kg b.wt.) for 28 days (Bello *et al*., 2016). Group 1 served as control and was orally administered with vehicle (normal saline) whereas group 2 and 3 was administered with *M. decumbens* methanolic root extract at oral dose level of 400 and 800 mg/kg b.wt/day, respectively for 28 days. Group 4 served as satellite group that received *Maerua decumbens* of 800 mg/kg body weight for 28 days but was continuously monitored untreated for 14 days after the end of the treatment to observe for any withdrawal symptoms, reversibility, persistence or delayed occurrence of toxic effects. Thus, the experimental groupings were as follows;

Group 1: Normal rats orally received normal saline daily;

Group 2: Rats treated orally with methanolic root extract at 400 mg/kg b.wt daily;

Group 3: Rats treated orally with methanolic root extract at 800mg/kg b.wt daily.

Group 4: Satellite group treated orally with methanolic root extract at 800mg/kg b.wt daily

3.8.2 Behavioral Changes, Body Weight and Mortality

During the entire dosing period, the animals were observed daily for clinical signs of toxicity on behavioral changes, morbidity and mortality. The rats were weighed (after

overnight fast) prior to dosing, after every 7 days, and before sacrifice on the last day in accordance with OECD guideline no. 407 (2008).

3.8.3 Sacrificing and Blood Sample Collection

After oral administration of the plant extract for 28 days, the animals were anaesthetized in desiccator containing cotton soaked in chloroform. Each anaesthetized animal was laid on a dissecting board and a pair of scissors used to open up the animal by cutting through vertical mid-line from neck to peritoneum (Osano et *al.,* 2016). Blood samples were collected through cardiac puncture. After general anesthesia with chloroform, a 22 gauge needle attached to a 3 ml syringe was inserted to the notch at the caudal aspect of the sternum and directed to the heart. The position was determined by palpating for the heartbeat. The plunger was pulled backwards gently in order to draw blood. The collected blood was divided into two portions; one for hematological analysis, which was collected in vacutainers containing anticoagulant ethylenediaminetetraacetic acid (EDTA) and the other for biochemical analysis, which was collected in tubes without anticoagulant. Blood for biochemical tests was kept for 30 minutes at room temperature to allow clotting. This was followed by centrifugation at 2500 revolutions per minute (rpm) for 10 minutes to obtain serum. The serum obtained was placed in Eppendorf tubes and stored at -20ºC awaiting biochemical analysis.

3.8.4 Collection of Tissues and Determination of Relative Organ Weights

The liver, kidneys, heart and spleen tisues were isolated by dissecting each organ from the sacrificed animals. Each organ was washed using ice cold normal saline to remove blood and then dried using blotting paper and weighed using analytical balance

(AUW220 Shimadzu Corporation, Japan). The relative organ weight of each animal was then calculated as follows:

Relative organ weight = absolute organ weight (g) × 100/animal fasting body weight on day of sacrifice (g).

Representative liver and kidney tissue sections were also washed in ice cold normal saline to remove blood and then fixed in 10% formalin for histopathology**.**

3.8.5 Determination of Serum Biochemical Parameters

Serum that was obtained as described in section 3.8.3 and kept at -20°C was thawed at room temperature before analysis of liver function indices (alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, total protein, and albumin) and kidney function indices (Urea and creatinine) using COBAS integra 400 plus auto-analyzer from Roche Company at Moi Teaching and Referral Hospital, Eldoret, Kenya. In brief, serum samples were allowed to thaw at room temperature for 30 minutes after removal from the deep freezer. From each serum sample, 150µL was pipetted into a sample cup, labeled and placed in a sample rack. The COBAS Integra 400 Plus contains an in vitro diagnostic reagent systems intended for use on COBAS systems for the quantitative determination of serum analytes. The sample rack with serum samples was inserted into the auto analyzer and commanded through a software system to run the liver and kidney function tests. The manufacturer's protocol was followed for all the test estimations. Printouts of the results were generated after 30 minutes. Serum analysis using the COBAS Integra 400 plus auto-analyzer is based on the principles described in the subsequent paragraphs for each of the test parameter that was determined.

For ALP, in the presence of magnesium and zinc ions, p-nitrophenyl phosphate is cleaved by phosphatases into phosphate and p-nitrophenol. The p-nitrophenol released is directly proportional to the catalytic activity of ALP. It is determined by measuring the increase in absorbance at 409 nm. For AST which catalyzes the reaction between L-aspartate and 2-oxoglutarate, the oxaloacetate formed in the reaction is reduced by NADH in a reaction catalyzed by malate dehydrogenase to form L-malate and NAD^+. The rate of the NADH oxidation is directly proportional to the catalytic activity of AST which is is determined by measuring the decrease in absorbance at 340 nm. For ALT that catalyzes the reaction between L-alanine and 2-oxoglutarate, the pyruvate formed in the reaction is reduced by NADH in a reaction catalyzed by lactate dehydrogenase to form L-lactate and NAD^+. The rate of the NADH oxidation is directly proportional to the catalytic activity of ALT which is determined by measuring the decrease in absorbance at 340 nm (Eraslan *et al*., 2007).

For total proteins; divalent copper reacts with the peptide bonds of proteins under alkaline conditions to form the characteristic pink to purple biuret complex. Sodium potassium tartrate prevents copper hydroxide precipitation and potassium iodide prevents the auto-reduction of copper. The color intensity is directly proportional to the protein concentration. It is determined by measuring the increase in absorbance at 552 nm. For albumin, at pH 4.1 it is sufficiently cationic to bind the anionic dye bromocresol green to form a blue-green colored complex. The intensity of the blue-green color is directly proportional to the concentration of albumin in the sample and is measured photometrically by monitoring the increase in absorbance at 583 nm (Ige *et al.,* 2015).

Urea is hydrolyzed by urease to form ammonium and carbonate. In the second reaction, 2-oxoglutarate reacts with ammonium in the presence of glutamate dehydrogenase and the coenzyme NADH to produce L-glutamate. In this reaction two moles of NADH are oxidized to NAD for each mole of urea hydrolyzed. The rate of decrease in the NADH concentrations is directly proportional to the urea concentration in the specimen. It is determined by measuring the absorbance at 340 nm (Sarkar, 2013). For creatinine, the enzymatic method is based on the established determination of hydrogen peroxide after conversion of creatinine with the aid of creatininase, creatinase, and sarcosine oxidase. The liberated hydrogen peroxide reacts with 4-aminophenazone and 2, 4, 6-triiodo-3-hydroxybenzoic acid to form a quinone imine chromogen. The color intensity of the quinone imine chromogen formed is directly proportional to the creatinine concentration. It is determined by measuring the increase in absorbance at 552 nm (Sharma *et al.*, 2004).

3.8.6 Determination of Hematological Parameters

Vacutainers containing the whole blood collected through cardiac puncture as described in section 3.8.3 was immediately kept in cool box and taken to Moi Teaching and Referral Hospital, for complete blood count. Parameters including white blood cell counts (WBC) and differential leucocyte count (neutrophils, lymphocytes, monocytes, eosinophil and basophils), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), corpuscular hemoglobin (CH), corpuscular hemoglobin concentration (CHC), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), red blood cell distribution width to corpuscular volume (RDW), Hemoglobin distribution width (HDW), mean platelet volume (MPV)

and platelets (PLT) were determined, using Advia 2120i hematology analyzer (Global Source Trading L.L.C Siemens) according to the manufacturer's instructions.

The ADVIA 2120i is a fully automated differential cell counter and consists of an analytical module that aspirates, dilutes, and analyzes whole blood samples; an auto sampler that automatically mixes, identifies, and presents samples for processing; a computer workstation that controls the instrument, provides primary user interface with the instrument and manages the data produced by the instrument; and a printer that optionally generates reports based on the instrument results (Harris *et al*., 2005; Davis & Barnes, 2012). Briefly, the blood was well mixed (though not shaken) and placed on a rack in the analyzer.

This instrument has flow cells, photometers and apertures that analyze different elements in the blood. The cell counting component counts the numbers and types of different cells within the blood. The principles for determination of hematological parameters are described below: WBC Count: The whole blood sample is mixed with ADVIA 2120i BASO reagent that contains acid and surfactant. The red blood cells are hemolyzed, and the white blood cells are then analyzed using two-angle laser light scatter signals. Red Blood Cell and Platelet Counts: Red blood cells and platelets are analyzed by a single optical cytometer after appropriate dilution of the blood sample with ADVIA 2120i RBC/PLT reagent (CBC TIMEPAC). The red blood cells are isovolumetrically sphered and lightly fixed to preserve the spherical shape. Red cells and platelets are counted from the signals from a common detector with two different gain settings.

Red Blood Cell and Platelet Size: Simultaneous measurement of laser light scattered at two different angular intervals is used to determine the size of red blood cells and

platelets. Hemoglobin: The hemoglobin method employs a modification of the manual cyanmethemoglobin method.

WBC Differential Method: The ADVIA 2120i utilizes the peroxidase and basophil/lobularity methods to quantitatively measure the following hematological parameters: neutrophils, lymphocytes, monocytes, eosinophils, large unstained cells, and basophils. With the peroxidase method, leukocytes are classified by the characteristic properties exhibited by cell-specific constituents when the cells are treated with cytochemical stains. With the basophil/lobularity method, red blood cells are hemolyzed and the cytoplasm is stripped from all white blood cells except basophils. The sample is then analyzed by two-angle laser light scattering detection using a laser diode. The white cells are classified into three categories: basophils, mononuclear (MN) cells, and polymorphonuclear (PMN) cells.

3.8.7 Histopathology of Liver and Kidney Tissues

The fixed liver and kidney tissues of representative groups which were obtained as described in section 3.8.4 were taken to Moi Teaching and Referral Hospital for tissue processing. After fixation, the specimens were trimmed appropriately using a scalpel to ensure they fitted into their respective labelled tissue cassettes. The filled cassettes were then stored in 10% formalin until further processing. The tissues were dehydrated by passing them in increasing concentrations of alcohol (70% to 95%) to remove water and formalin. A clearing agent (xylene) was used to remove the alcohol and was preferred because it allows infiltration with paraffin wax. The specimens were then infiltrated with an embedding agent (paraffin wax) and allowed to solidify to form blocks. These blocks provide a support matrix that allows thin sectioning. Wax was removed from the surface

of the block to expose the tissue. The blocks were chilled on a refrigerated plate for about 10 minutes before sectioning. A microtome (SLEE medical model GmbH) was used to slice extremely thin tissue sections off the block in the form of a ribbon. The microtome was pre-set to cut the tissues at thicknesses of 5 µm. The tissue ribbons were thereafter floated on a warm water bath to remove wrinkles. The tissue ribbons were then picked up on a glass microscopic slide. The glass slides were then placed in a warm oven at 37ºC for about 15 minutes to help the section adhere to the slide. Before staining, the slides were "deparaffinised" by passing them through xylene to alcohol to water.

Haematoxylin and eosin stains were used to provide contrast to tissue sections, making tissue structures more visible and easier to evaluate. After staining, cover slips were mounted over the tissue specimens on the slides using optical grade glue, to help protect the specimen. The stained slides were finally examined and interpreted by a pathologist, Dr, Benson Macharia of Department of Human Pathology and Forensic Medicine, School of Medicine, Moi University, Kenya for histological changes under a light microscope (Olympus CX21FS1). Examination under the microscope was followed by taking photomicrographs using Redmi Note 4 model 2016102 phone from Xiaomi communication Company Limited, China. The microscopic features of the liver and kidney of male and female rats were compared with those of their corresponding control groups.

3.9 Antidiabetic Activity Study of Root Extract in Rats

3.9.1 Streptozotocin (STZ)-induced Type 1 Diabetes Model

Streptozotocin diabetes-induced model was used to evaluate the antidiabetic activity of the extract. Rats were initially handled as described in section 3.6. A total of 30 male rats aged 6-8 weeks, and of body weight range of 140-180 g were used in this study. The rats were fasted overnight before the administration of 50 mg/kg b.wt of STZ for diabetes induction. Diabetes was induced in rats by single intraperitoneal injection of STZ dissolved in cold 0.1 M sodium citrate buffer of pH 4.5. Five rats for normal control group and 5 normal rats for administration of the extract only were all injected intraperitoneally with 0.2 mL of vehicle (0.1M sodium citrate buffer). After the injections, the animals were allowed to drink 5% glucose solution overnight to overcome hypoglycemic shock and the rats had free access to food and drinking water. The fasting blood glucose level was measured after 5 days using glucometer. Rats with glucose levels above 13.9mmol/L were considered as diabetic and used for further experimentation.

3.9.2 Experimental Design and Treatment Groups

After successful diabetic induction in rats, 20 diabetic and 10 normal male rats were randomly divided into 4 groups and 2 groups respectively with each group having 5 male rats. The vehicle (normal saline) was administered to each rat at 1ml/kg b.wt. The experimental designed was based on the experiment by Olatunde *et al*. (2014) with minor modifications. The experimental grouping and its treatment was as detailed below;

Group 1: Normal control rats on normal saline;

Group 2: Normal rats but treated with *M. decumbens* extract at 400 mg/kg b.wt daily;

Group 3: Diabetic control rats untreated on normal saline;

Group 4: (Low dose): Diabetic rats treated with *M. decumbens* extract at 100 mg/kg b.wt daily;

Group 5: (High dose): Diabetic rats treated with *M. decumbens* extract at 400 mg/kg b.wt daily;

Group 6: (Standard): Diabetic rats treated with metformin at 100 mg/kg b.wt daily.

The vehicle (normal saline), metformin and *M. decumbens* extracts were administered to respective groups via the oral route at regular intervals daily for 21 days. Signs of diabetes mellitus were physically monitored in the STZ-induced diabetic rats. Rats had free access to food and drinking water *ad libitum* except when measurements of fasting body weights and fasting blood glucose were being assessed whereby the rats were fasted overnight but with water *ad libitum*. These fasting body weights and blood glucose were determined weekly that is, on days 0, 7, 14, and 21 of the study.

3.9.3 Sacrifice and Samples Collection

The rats were fasted overnight prior to terminal sacrifice. On the 21st day, the rats were weighed, fasting blood glucose from tail vein were determined then the rats were anaesthetized with chloroform before tissue collection. All the rats were then dissected to collect the liver and pancreas. Liver and pancreas tissues sections were washed in ice cold normal saline to remove blood and then fixed in 10% formalin for histopathology**.** Liver sections for determination of MDA levels as an index of lipid peroxidation were also cut and immediately washed in ice cold normal saline, placed in labeled polypots and stored at -20 ^{0}C until the time for analyses.

3.9.4 Determination of Liver Malondialdehyde Levels

Lipid peroxidation was estimated by the measurement of levels of malondialdehyde (MDA) in liver tissues. MDA level in the liver tissue samples was measured using the reaction with thiobarbituric acid as described by Ohkawa *et al*. (1979). Briefly, liver homogenate was prepared in a ratio of 1 g of tissue to 9 ml of 1.15% of cold potassium chloride and homogenized and centrifuged at 2000 rpm for 10 minutes. Then 0.1 ml of supernatant was pipetted and added 0.2 ml of 8.1% SDS, 1.5 ml of 20% acetic acid solution and the pH adjusted to pH 3.5 with 10 M NaOH, and 1.5 ml of 0.8% aqueous solution of TBA was added. The mixture was made up to 4 ml with distilled water, and then heated at 95°C for 60 minutes in a water bath. After cooling with tap water, 1 ml of distilled water was added and the red pigment produced was extracted with 5 ml of the mixture of n-butanol and pyridine (15: 1, v/v) and shaken vigorously. After centrifugation at 4000 rpm for 10 minutes, the pink organic layer was pipetted out without disturbing the bottom and its absorbance was read at 532nm using spectrophotometer (Spectronic 21D, Milton Roy). Tetramethoxypropane (TMP) was used as an external standard and was prepared as follows; from stock concentration of TMP 990 g/L (6.03 M), 100mM solution was made by taking 1666 µl of TMP diluted to 100ml with distilled water. This solution was used to make 100 µM stock solutions by taking 50 µL of 100 mM solution and diluting to 50ml with distilled water. This stock solution was used to make series of 0.5 µM, 1 µM, 2 µM, 3 µM, 4 µM, 5 µM, 7.5 µM and 10 µM standard solutions. The micromolar solutions were used to prepare the MDA standard curve (Appendix II) in nM (5 nM to 100 nM) by pipetting 0.1 ml of each respective µM solution and diluted to 10 ml by treating the same way as the sample. The reference blank was of 1.15% KCl and

treated in the same way as the samples and standards by addition of the same reagents. The levels of MDA were expressed in nM.

3.9.5 Histopathology of Liver and Pancreas Tissues

The fixed tissues of liver and pancreas of representative groups were obtained as described in section 3.9.3 and histopathological assays were done as described earlier in section 3.8.7.

3.10 Data Management and Statistical Analysis

The data was entered in the Microsoft Excel Spread Sheet (Version 2010). Data of mean body weights, organ weights, biochemistry and hematology parameters and MDA results were statistically analyzed using one-way analysis of variance (ANOVA) while all other data were subjected to Student t-test. Results were expressed as mean ± standard error of mean (SEM). Values were considered significantly different at a confidence level of 95% *($p < 0.05$).*

CHAPTER FOUR

RESULTS

4.1 Qualitative Phytochemical Screening of *Maerua decumbens* Root Extract

The qualitative phytochemical screening of *M. decumbens* methanolic root extract showed the presence of alkaloids, glycosides, flavonoids, phenolic compounds, saponins, steroids, tannins and terpenoids, while anthraquinones were not detected (Table 4.1).

Table 4.1: Qualitative Phytochemical Screening of *Maerua decumbens* Root Extract

Phytochemicals	Result
Alkaloids	+
Anthraquinones	-
Glycosides	+
Flavonoids	+
Phenols	+
Saponins	+
Steroids	+
Tannins	+
Terpenoids	+

***'+'** indicates present, '-'indicates absent.*

4.2 Acute Oral Toxicity of *Maerua decumbens* Root Extract in Female Rats

It was observed that the methanolic extract of the roots of *Maerua decumbens* at 2000 mg/kg b.wt did not induce changes in the behavior of female rats during the first 30 min and for a period of up to 4 h after oral administration. All the animals rubbed their mouth

and nose with their front pawns and against the walls of the cage soon after dosing. All these symptoms disappeared completely after 30 minute post dosing. The extract did not cause diarrhea but the droppings in some test animals were watery and not well formed like pellets. There was no death during acute oral toxicity testing of *Maerua decumbens* root extract at 2000 mg/kg b.wt.

Body weight changes during the 14 days treatment days for the three animals dosed at 2000 mg/kg b.wt increased steadily (Table 4.2). All extracts treated animals showed a stable increase in body weights during the 14 days. Therefore, the approximate acute lethal dose (LD_{50}) of *M. decumbens* extract in female rats was estimated to be higher than 2000 mg/kg b.wt in female rats.

Table 4.2: Effect of Acute Oral Toxicity of *Maerua decumbens* Extract on Body Weight in Rats

	Body Weights (g)			**% Body weight gain on 14th day (from Day 0)**
	Duration (Days)			
	0	**7**	**14**	
Normal Control	194.70±18.00	202.59±16.97	205.25±16.48	10.5%
2000 mg/kg b.wt	155.57±3.62	169.55±0.98	180.19±1.64	16%

Results are expressed as mean ±SEM. (n=3)

4.3 Sub-Acute Oral Toxicity of *Maerua decumbens* Root Extract in Rats

4.3.1 Clinical Signs of Toxicity

Daily oral administration of the animals at 400 mg/kg b.wt and 800 mg/kg b.wt dose of *M. decumbens* root extract for 28 days did not induce any obvious symptom of toxicity in rats of both sexes, including the highest dose tested at 800mg/kg body weight. Transient clinical signs that were most pronounced after dosing and lasted for about 30 minutes included raised fur and rubbing at the oral cavity indicating irritation at any time treatments were administered. No deaths or additional signs of toxicity were found in any groups throughout the experimental period. Physical observation of the treated rats throughout the study indicated that none of them showed signs of toxicity in their skin, fur, eyes, mucus membrane, or behavioral changes, diarrhea, tremors, salivation, sleep, and coma. The satellite group was observed daily for the next 14 days beyond day 28 days of daily treatment with 800 mg/kg of root extract. Animals did not show any delayed behavioral signs of toxicity and there was normal increase in body weight over the observation period (Appendix I).

4.3.2 Effect of Root Extract on Body Weight Changes

There was a steady gain in body weights among all treated groups as well as the control group from start to the end of the 28 day repeated oral dose study (Table 4.3). The percent change in body weights between day 0 and day 21 were such that there was steady increase in body weights in all the extract treated groups (Table 4.3). As compared to untreated controls, the *M. decumbens* root extract seems to enhance increase in the body weights in all the extract treated rats at both low and high doses.

Table 4.3: Body Weight Changes of Rats in Sub-Acute Oral Toxicity Study of Extract of *M. decumbens*

	Body Weight (g)					
	Duration (Days)					% Change in b.wt on 28th Day (from Day 0)
	0	7	14	21	28	
Treatment						
Male						
Normal control	150.55±8.89	183.75±3.57	192.08±2.99	204.96±4.56	207.95±8.54	38.1
Low dose (400 mg/kg)	79.30±1.68	104.38±2.05	124.88±2.56	137.92±2.16	164.09±2.46	106.9
High dose (800 mg/kg)	106.44±7.54	140.20±4.42	147.24±4.54	174.28±4.81	201.11±2.57	88.9
Female						
Normal control	143.35±8.64	163.84±9.23	175.27±6.09	184.18±4.31	183.71±4.30	28.2
Low dose (400 mg/kg)	101.72±11.16	137.24±14.00	156.25±13.17	163.08±11.54	188.83±10.31	85.6
High dose (800 mg/kg)	121.46±8.79	150.89±11.84	159.64±11.80	176.35±13.05	198.14±13.80	63.1

Results are expressed as mean ±SEM. (n=5)

4.3.3 Effect of Root *Maerua decumbens* Extract on Relative Organ Weights of Rats

Relative organ weights of *M. decumbens* treated rats and control rats following Sub-acute oral administration are shown in Table 4.4. The relative organ weights of organs recorded at necropsy in the treatment groups did not show a significant difference compared to the control in the liver at doses of 400 and 800 mg/kg b.wt of extract in both genders. There seem to be incidental and dose unrelated significant decreases and/or increases of relative organ weights of kidney (increase in low dose male and high dose female), heart (reduced in low dose male and female) and spleen (increased in high dose male and reduced in high dose female) in extract administered treated rats (Table 4.4).

Table 4.4: Effect of *M. decumbens* Extract on Relative Organs Weight of Rats in Sub-acute Oral Toxicity

	Relative Organ Weight (%)		
Organ	**Control**	**400 mg/kg b.wt**	**800 mg/kg b.wt**
Male			
Liver	3.60±0.28	4.13±0.25	4.34±0.16
Kidney	0.81±0.03	0.84±0.01[a]	0.83±0.02
Heart	0.43±0.01	0.39±0.01[a]	0.42±0.01
Spleen	0.38±0.01	0.55±0.03	0.50±0.04[a]
Female			
Liver	4.54±0.32	4.07±0.13	4.73±0.27
Kidney	0.79±0.04	0.78±0.04	0.85±0.01[a]
Heart	0.45±0.00	0.39±0.02[a]	0.45±0.02
Spleen	0.45±0.00	0.53±0.03[a]	0.41±0.04[a]

Values are expressed as mean ± SEM. (n=5*). [a]P <0.05 significantly different compared to control*

4.3.4 Effect of *Maerua decumbens* Root Extract on Serum Biochemical Parameters

The effects of sub-acute oral administration of *M. decumbens* methanolic root extract on serum biochemical parameters are presented in Tables 4.5. The *M. decumbens* methanolic root extract had no statistically significant difference *($p<0.05$)* on the liver function parameters that is alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in all dosage groups in male and female rats compared to their respective normal control groups at $p<0.05$. There was no significant change in albumin in all treatment groups compared to respective normal controls. Total protein in both female (400 and 800 mg/kg) and male (800 mg/kg) group had significant reduction compared to respective controls (Table 4.5). In the kidney function parameters; urea, was significantly reduced in male and female groups at 800 mg/kg dose (30.77% and 25.96%) respectively compared to corresponding normal controls, while creatinine had significant reduction in female at 800 mg/kg dose (18.90%) compared to its respective control at $p<0.05$ (Table 4.5).

Table 4.5: Effect of *M. decumbens* Root Extract on Serum biochemical Parameters in Rats

	Male			Female		
Parameter	**Control**	**400 mg/kg b.wt**	**800 mg/kg b.wt**	**Control**	**400 mg/kg b.wt**	**800 mg/kg b.wt**
ALP (U/L)	182.08±13.64	179.43±8.56	223.03±15.61	109.10±22.17	93.17±13.51	177.20±17.94
AST (U/L)	119.18±11.65	162.45±5.51	154.84±11.84	144.72±10.09	163.97±6.21	159.25±15.60
ALT (U/L)	64.76±12.12	80.16±2.15	99.62±5.66	84.96±15.57	81.80±5.29	92.36±11.26
TP (g/L)	68.54±1.28	64.78±0.76	63.06±1.35[a]	72.22±1.92	66.00±1.38[a]	63.60±1.74[a]
ALB (g/L)	35.57±1.28	36.11±0.69	35.12±0.74	37.26±1.14	37.12±0.64	35.44±2.30
Urea(mmol/L)	10.40±1.06	9.63±0.30	7.21±0.27[a]	9.90±0.77	9.28±0.64	7.33±0.58[a]
CRE (µmol/L)	33.20±3.12	27.80±1.16	31.40±2.77	32.80±2.03	31.50±1.58	26.60±0.93[a]

Values are expressed as mean ± SEM (n=5). Means followed by superscript "[a]" across rows are significantly different at p<0.05 as compared to the corresponding control. ALP-alkaline phosphatase, ALT-alanine aminotransferase, AST-aspartate aminotransferase, TP-total protein, ALB-albumin, CRE-creatinine.

4.3.5 Effect of *Maerua decumbens* Root Extract on Hematological Parameters

The effects of sub-acute oral administration of *M. decumbens* methanolic root extract on hematological parameters in rats are presented in Tables 4.6. The hematological parameters measured (neutrophils, lymphocytes, monocytes, basophils, platelet count and CHC) in treated rats showed no significant difference from their respective controls. However, there were afew alterations that appear not to be dose dependent and gender specific significant variations in certain parameters as WBC, eosinophils, hemoglobin, RDW, HDW, HCT, MCHC, HGB, MCV, MCH, CH and MPV ($P < 0.05$) as shown below in Table 4.6.

Table 4.6: Effect of *M. decumbens* Root Extract on Hematological Parameters in Rats

	Male			Female		
Parameter	**Control**	**400 mg/kg b.wt**	**800 mg/kg b.wt**	**Control**	**400 mg/kg b.wt**	**800 mg/kg b.wt**
WBC (10^{-3}/µL	3.05±0.97	5.45±0.85	6.98±0.45[a]	4.82±0.98	5.86±0.79	4.42±1.17
Neutrophils (%)	10.03±2.97	12.96±3.17	10.30±4.72	16.58±5.78	11.30±3.40	10.23±1.12
Lymphocyte (%)	34.23±1.94	46.80±8.05	48.36±8.56	45.98±2.71	55.26±4.70	54.92±7.17
Monocyte (%)	25.65±3.23	26.54±4.41	17.10±1.50	30.60±7.20	20.98±2.15	24.08±1.94
Eosinophil (%)	1.05±0.29	2.88±0.21[a]	0.65±0.23	4.50±1.62	1.72±0.63	0.60±0.17[a]
Basophil (%)	0.84±0.14	0.66±0.25	1.22±0.23	0.68±0.22	0.60±0.10	1.30±0.59
RBC (10^{-6}/µL	8.67±0.30	7.83±0.24[a]	7.13±0.47[a]	8.39±0.27	6.79±0.28[a]	6.91±0.28[a]
HGB (g/dL)	15.78±0.58	14.66±0.34[a]	14.08±0.90	15.02±0.26	13.18±0.59[a]	13.70±0.29[a]
HCT (%)	49.10±2.06	47.56±1.26	43.52±2.45	47.60±1.13	41.40±1.81[a]	41.78±1.23[a]
MCV (fL)	56.58±0.51	60.74±0.66[a]	61.36±2.14[a]	56.84±1.05	60.94±0.75[a]	60.68±1.81[a]
MCH (pg)	18.20±0.38	18.74±0.24	19.76±0.36[a]	17.96±0.40	19.40±0.25[a]	19.92±0.54[a]
MCHC (g/dL)	32.16±0.57	30.84±0.16[a]	32.26±0.71	31.58±0.37	31.84±0.67	32.86±0.52[a]
CHC (g/dL)	29.74±0.42	28.34±0.09	29.86±0.48[a]	29.44±0.40	29.56±0.45	30.34±0.43
CH (pg)	16.82±0.15	17.22±0.17	18.28±0.38[a]	16.70±0.44	17.98±0.22[a]	18.36±0.49[a]
RDW (%)	13.92±0.21	12.78±0.26	14.30±0.71[a]	14.18±1.06	13.70±0.56	13.68±0.20
HDW (g/dL)	2.81±0.05	2.54±0.04	2.74±0.10[a]	2.69±0.08	2.55±0.06	2.69±0.04
PLT (10^{-3}/µL)	351.67±28.66	332.33±20.85	354.40±26.79	423.25±59.23	306.75±33.92	347.00±43.97
MPV (fL)	8.68±0.19	9.44±0.21[a]	9.38±0.20[a]	8.84±0.19	9.38±0.10[a]	9.34±0.18[a]

Values are expressed as mean ± SEM (n=5). Means followed by superscript "[a]" across rows are significantly different at $p < 0.05$ as compared to the corresponding control. WBC-white blood cells, RBC-red blood cells, HGB-hemoglobin, HCT-hematocrit, MCV-mean corpuscular volume, MCH-mean corpuscular hemoglobin, MCHC-mean corpuscular hemoglobin concentration, CHC-corpuscular hemoglobin concentration, CH-corpuscular hemoglobin, RDW-red cell distribution width, HDW- hemoglobin distribution width, PLT-platelets, MPV-mean platelet volume

4.3.6 Effect of *Maerua decumbens* Root Extract on Rat Liver and Kidney Histology

Light microscopic examination of sections of liver and kidney of control and *M. decumbens* extract administered groups showed absence of any gross and microscopic lesions (Figure 4.1 and 4.2). Histopathological section of liver in representative control group and *M. decumbens* extract treated male and female groups at a dose of 400 and 800 mg/kg for 28 days showed normal liver architecture (Figure 4.1). The portal triads and central veins in liver tissues were found to be normal in both control and extract administered rats (4.1). There was no evidence of toxic signs observed in the liver tissues as there were no inflammations in all the treatment groups (Figure 4.1). Kidney histopathology sections for normal control group and extract treated groups at doses of 400 and 800 mg/kg also showed normal architecture (Figure 4.2).

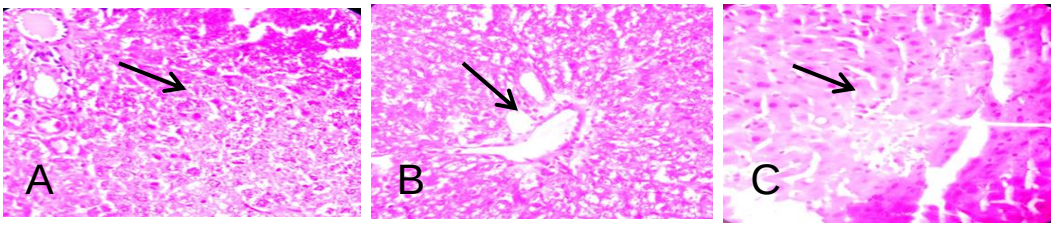

Figure 4.1: Photomicrographs of the Liver Sections of Representative Groups:
A- normal control, **B**-400mg/kg and **C**-800mg/kg *Maerua decumbens* root extract treated rats. *Arrows show the normal histoarchitecture; A- normal hepatic architecture; B- the portal triad C- normal hepatocytes (Magnification 40x).*

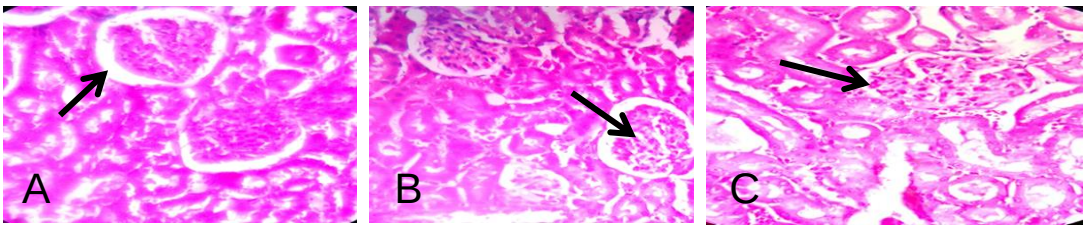

Figure 4.2: Photomicrographs of the Kidney Sections of Representative Groups:
A- Normal control; **B**-400mg/kg Extract and **C**-800mg/kg Extract. *Arrows show the normal histoarchitecture; A- the Bowman's capsule, B- the glomeruli C- the renal tubules (Magnification 40x).*

4.4 Antidiabetic Activity of *Maerua decumbens* Extract in Rats

4.4.1 Effect of *Maerua decumbens* Root Extract on Fasting Body Weight

In order to monitor the effect of methanolic root extract of *Maerua decumbens* on body weight in diabetic rats, the fasting body weight of each animal was recorded on 0, 7^{th}, 14^{th} and 21^{st} day of treatment. There was a comparable increase in body weight observed in normal control rats versus normal rats treated with the methanolic root extract (28.73% and 35.23% respectively) after 21 days of treatment compared to day 0. There was however weight decrease (15.32%) in diabetic untreated group after 21 days from day 0. Body weights of the animals in metformin-treated group (standard drug) also marginally increased (2.12%) after 21 days of treatment from day 0 **(**Table 4.7). Treatment of diabetic rats with methanolic root extract of *Maerua decumbens* (100 mg/kg b.wt and 400 mg/kg b.wt) marginally improved the weight gain (1.97% and 3.69% respectively) after 21 days of treatment.

Table 4.7: Effect of *M. decumbens* Extract on Body Weights in Diabetic and Normal Rats

Body weights (g)						% change in body weight on 21st Day (from Day 0)
	Duration (Days)					
Treatment	**Baseline**	**0**	**7**	**14**	**21**	
Normal control	145.40±2.66	160.26±3.72	173.08±4.75	192.78±5.33	206.30±9.04	28.73
Diabetic control	178.60±0.66	177.40±1.60	168.52±3.45	157.10±2.66	150.22±4.97	-15.32
Normal rats + 400 mg/kg extract	148.86±4.54	156.92±4.50	174.18±7.00	197.58±10.14	212.18±11.40	35.23
Diabetic rats + 100 mg/kg extract	163.82±6.44	164.64±6.62	164.74±6.97	169.16±6.72	167.88±6.57	1.97
Diabetic rats + 400 mg/kg extract	176.18±1.48	175.64±1.58	181.02±5.30	186.10±6.76	182.12±6.90	3.69
Diabetic rats + metformin (100 mg/kg)	163.70±4.41	162.06±5.12	162.96±7.31	163.42±11.56	165.54±13.98	2.12

Values are expressed as Mean ± SEM (n = 5).

4.4.2 Effect of *Maerua decumbens* Extract on Fasting Blood Glucose in Rats

At baseline, before induction of diabetes, fasting blood glucose levels were not significantly different in the various experimental groups, with mean normal values ranging between 4.0 and 5.0 mmol/L (Table 4.8). At Day 0 i.e five days after administration of streptozotocin (50 mg/kg b.wt), diabetic induced rats displayed significant hyperglycemia with mean values of fasting blood sugar of between 24.16 mmol/L and 26.60 mmol/L (Table 4.8). Oral administration of *M. decumbens* root extract for 21 days resulted in dose dependent significant reduction of fasting blood glucose (Table 4.8) compared to diabetic untreated control. The root extract at high dosage of 400 mg/kg b.wt produced the maximum fall of 62.12% from day 0 in the blood glucose levels in diabetic rats after 21 days of treatment as compared to diabetic control, while low dose of extract (100 mg/kg) had 45.94% reduction of fasting blood sugar levels. The standard drug, meformin at 100 mg/kg b.wt resulted in a (58.16%) reduction in fasting blood glucose from day 0.

Table 4.8: Effect of *M. decumbens* Root Extracton on Fasting Blood Glucose in Diabetic and Normal Rats

	Fasting blood glucose (mmol/L)					% change in blood sugar on 21st Day (from Day 0)
	Duration (Days)					
Treatment	**Baseline**	**0**	**7**	**14**	**21**	
Normal control	4.1±0.25	5.10±0.42[a]	4.64±0.34[a]	5.68±0.19[a]	5.86±0.17[a]	-
Diabetic control	5.0±0.30	24.16±2.47[b]	25.92±2.18[c]	19.94±1.33[d]	21.98±1.95[d]	-
Normal rats + 400 mg/kg	4.42±0.37	4.86±0.34[a]	4.70±0.14[a]	6.20±0.18[a]	5.76±0.18[a]	-
Diabetic rats + 100 mg/kg	4.0±0.19	26.60±2.59[b]	22.46±4.07[b]	15.86±2.21[c]	14.38±1.95[c]	-45.94
Diabetic rats + 400 mg/kg	4.42±0.14	25.46±3.19b	16.32±3.12[b]	11.70±2.92[b]	8.02±1.68[b]	-62.12
Diabetic rats + metformin	5.0±0.30	25.48±2.01[b]	19.90±1.93[b]	14.34±2.21[b]	10.66±2.20[b]	-58.16

Values are expressed as mean ±SEM (n=5). Means followed by different superscript within a column are significantly different at p<0.05 compared to normal and diabetic untreated control

4.4.3 Effect of *Maerua decumbens* Root Extract on Liver MDA Levels in Diabetic Rats

The levels of MDA, an index of lipid peroxidation in the liver tissues of experimental rats are presented in figure 4.3. There was significant *(p <0.05)* increase in the MDA levers in liver tissues of diabetic untreated control rats (838.46±44.52 nM) compared to normal control rats (503.85±24.63 nM). Treatment of diabetic rats with metformin (100 mg/kg b.wt), *M. decumbens* methanolic extract at 100 and 400 mg/kg b.wt caused a significant *(p<0.05)* reduction in the levels of liver MDA (500±47.5, 503.85±33.53 and 407.69±28.13 nM), respectively, compared to the diabetic untreated rats (838.46±44.52). There was also significant *(p <0.05)* reduction in liver MDA observed in the normal rats treated with extract at 400 mg/kg (300±30.77) compared to normal untreated rats (503.85±24.63).

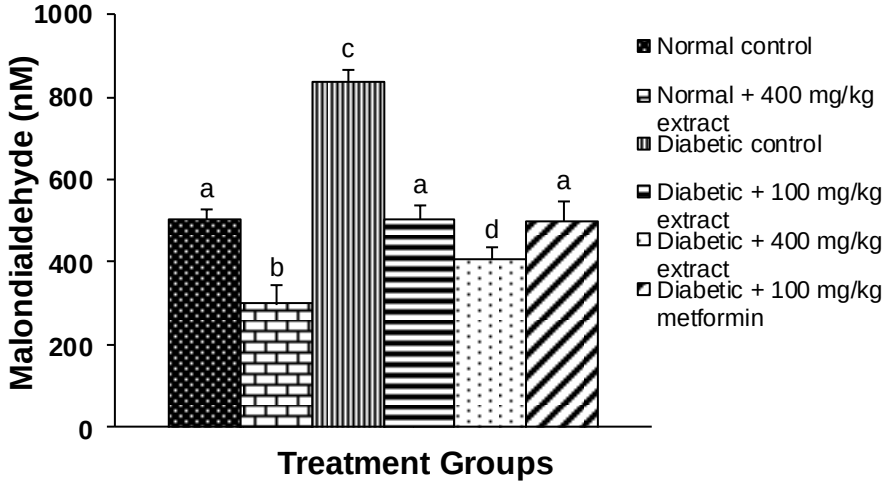

Figure 4.3: Malondialdehyde (MDA) Levels in Liver of Normal and Diabetic Rats.
Values are expressed as Mean±SEM (n=5). Bars with different letters are significantly different at p<0.05 compared to normal and diabetic control.

4.4.4 Effects of *Maerua decumbens* Root Extract on Liver and Pancreas Histology

The hematoxylin and eosin (H/E) stained liver sections of normal control groups (normal control-Figure 2-A and normal but extract treated group (400 mg/kg b.wt) - Figure 2-B) showed normal liver histology. The H/E staining of liver sections of the diabetic untreated control rats showed hepatic cells with mild periportal inflammation (Figure 2-C). The root extract (100 and 400 mg/kg b.wt) treatment preserved the hepatic cellular arrangements and reduced the inflammation in the liver of diabetic rats, although low dose treated rats had mild periportal inflammation (Figure 2-D & E). Treatment with metformin at 100 mg/kg.b.w also preserved hepatic histoarchitecture of the rats (Figure 2-F).

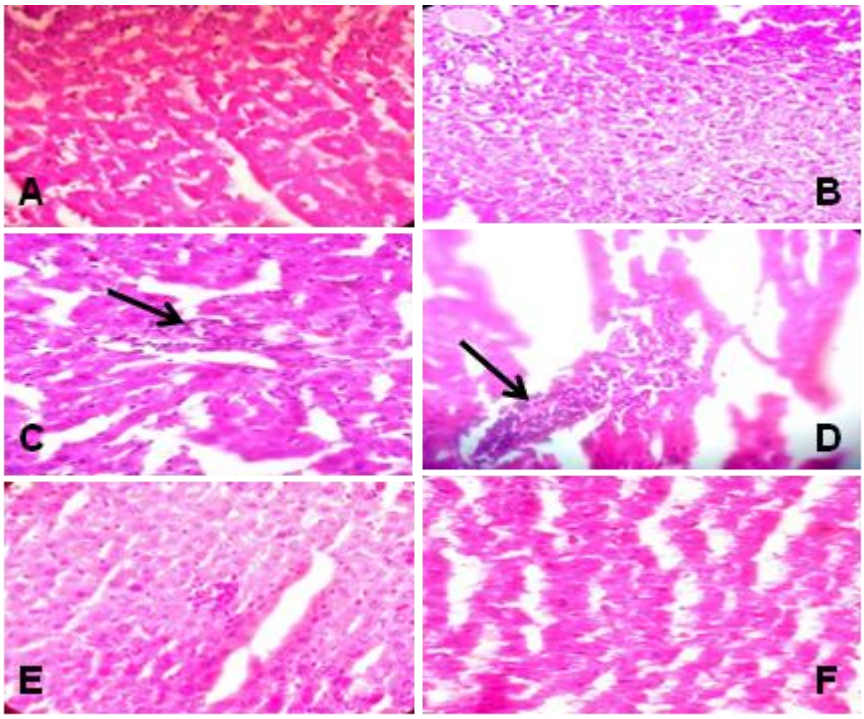

Figure 4.4: Photomicrographs of Liver Sections of Normal and Diabetic Rats:
A- Normal control rats; **B**-Normal-extract-treated rats (400 mg/kg); **C**- Diabetic control rats; **D**-Diabetic-extract treated rats (100mg/kg); **E**-Diabetic-extract treated rats (400mg/kg) and **F**-Diabetic-metformin treated rats (100 mg/kg). *Arrow: A - Normal liver histology; B-normal liver histology; C- Diabetic liver shows mild periportal inflammation; D- mild focal periportal inflammation; E- normal hepatic cells; F- Preserved hepatocytes; (Magnification 40x).*

The light microscopic examination by specific staining of pancreas sections of normal control (normal group and normal but extract (400mg/kg b.wt) treated group) showed presence of islets of Langerhans (Figure 4.5-A & B) which is not observed in the pancreas of STZ-induced untreated diabetic rats (Figure 4.5-C). In the *M. decumbens* root extract (100 and 400mg/kg b.wt) treated rats, the pancreas appear to have some preserved islets (Figure 4.5-D & E) similar to the normal control group. Metformin treatment also improved the deleterious effects of STZ on the pancreas as no islets were observed (Figure 4.5-F).

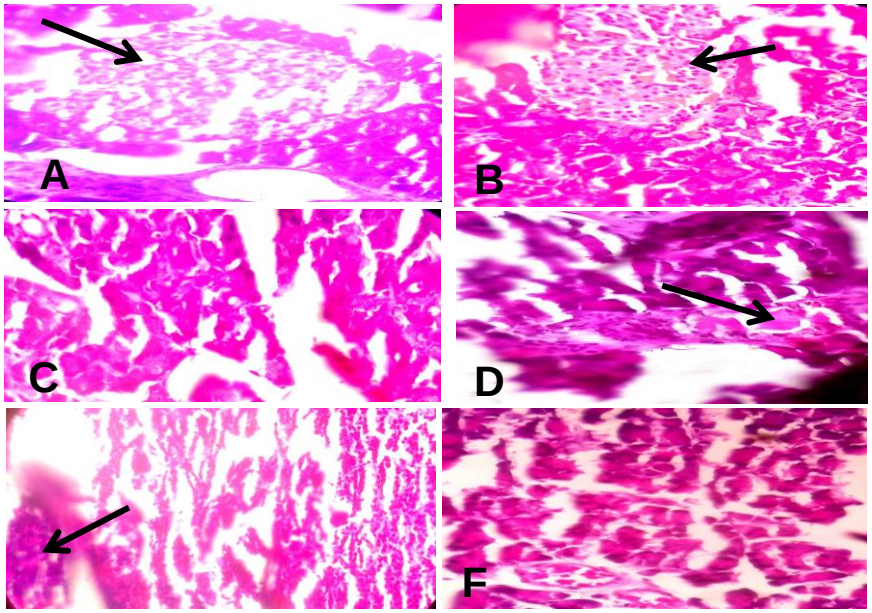

Figure 4.5: Photomicrographs of Pancreas Sections of Normal and Experimental Rats:
A-Normal control rats; **B**-Normal-extract-treated rats (400mg/kg); **C**- Diabetic control rats; **D**-Diabetic-extract treated rats (100mg/kg); **E**-Diabetic-extract treated rats (400 mg/kg) and **F**-Diabetic-metformin treated rats (100 mg/kg). *Arrows; A - Pancreas showing islets; B- Pancreas showing islets; C - Shows no islets; D- Shows reduced number of islets; E - Reduced islets; F-Shows no islets (Magnification 40x).*

CHAPTER FIVE

DISCUSSION

Various common herbs have been shown to contain powerful phytochemical compounds that can improve the quality of our health by protecting us against many diseases. Phytochemicals are bioactive, non-nutrient, naturally occurring plant compounds which are mostly used for medicinal purposes because of their medicinal value (Okarter *et al*., 2010). In this study, the results of the phytochemical screening of *Maerua decumbens* methanolic root extract showed the presence of flavonoids, tannins, saponins, alkaloids, glycosides, phenols and terpenoids (Table 4.1) but anthraquinones were not detected. The results of this study are in agreement to earlier study by Kiswii *et al.* (2014) that revealed the presence of flavonoids and cardiac glycoside**s** from their phytochemical analysis of *M. decumbens* leaf extracts. These phytochemicals exhibit various pharmacological and biochemical actions. Plants used in the treatment of diseases are said to contain bioactive components with biological activity some of which are responsible for the characteristic odor, pungencies and color of plant, while others give the particular plant its medicinal or poisonous virtue (Evans, 2009).

The presence of many bioactive compounds with mechanisms of action similar to conventional synthetic drugs could predict the potential toxic and adverse effects of phytomedicines (Ogbonnia *et al*., 2009). Animal toxicity testing studies have also shown that many plants currently used for phytotherapy, and just to name a few; *Momordica charantia*, *Urtica dioica*, *Crocus sativus* and *Erythrophleum guineese* (Prabu *et al.*, 2013), are highly toxic when given either acutely or sub-acutely. This necessitates the conduct of meticulously planned toxicity studies based on stipulated guidelines to assure

the safety of medicinal plants used in various traditional practices (Ogbonnia *et al.*, 2009).

The measurable endpoints in toxicity studies may be a pharmacological, biochemical, or a pathological change, which shows percentage or proportional change. Alternatively, the endpoint of toxicity may be an all-or-none or quantal type of effect such as death or loss of consciousness (Timbrell, 2009). There are many biomarkers of response, which can be measured. These include markers such as enzymes, which appear in the blood when an organ is damaged, increases in enzymes or stress proteins resulting from induction changes in urinary constituents resulting from damage or metabolic dysfunction, increases or decreases in enzyme activity, hematological and pathological changes detected at the gross, microscopic, and subcellular level. Indeed, a biomarker of response could be almost any indication of altered structure or function of the cell (Timbrell, 2009).

The present study evaluated the toxicity of oral administration of the methanolic extract of *Maerua decumbens* root in rats. The acute oral toxicity study conducted at the limit dose of 2000mg/kg was found to be well tolerated. Single-dose oral administration of *M. decumbens* in female rats at 2000 mg/kg b.wt had no mortality effects, observed clinical signs and body weight chnages as compared to normal control (Table 4.2). Therefore, no acute toxicity was found in rats treated with *M. decumbens* and the approximate lethal dose was determined to be higher than 2000 mg/kg body weight.

This study also evaluated the sub-acute oral toxicity of administration of the methanolic extract of *Maerua decumbens* root in rats for 28 days. Both sexes of the animals were allocated in each group because toxicological studies have shown small differences in

sensitivity between females and males (OECD, 2001). The rats administered root extract of *Maerua decumbens* orally at daily doses of 400 and 800 mg/kg b.wt, demonstrated no changes in animal behavior such as salivation, diarrhea, change of fur or convulsions, as well as increase in body weights in both male and female rats at low and high doses (Figure 4.3). In toxicity studies, changes in body weight have been used as an indicator of adverse effects of drugs and chemicals. It goes without saying that a decrease in body weight may be an indicator of adverse effects (Tahraoui *et al.,* 2010). However, in this study there was stable increase in body weights of all male and female treated rats at all doses (Table 4.3). The increase in body weight observed during treatment at the high oral dose confirms the nontoxicity of the extract. The vital organs were also weighed during sacrifice day. The significance of weighing organs takes account of their sensitivity to envisage toxicity, enzyme stimulation, physiologic perturbations, and acute injury (Michael *et al*., 2007). The findings of this sub-acute study showed that the vital organs (liver, kidneys, heart and spleen) were not adversely affected for toxicity throughout the treatment in both male and female treatment groups except incidental significant increases and/or decreases in the kidney, heart and spleen which were non-dose related and could be due to handling inconsistencies of the organs, and therefore, this implies that the presence of the extract in the animal and the various oral concentrations used during the test had little or no impact on the particular organs and provides support on the safety of *M. decumbens* root extract (Table 4.4). Moreover, the satellite group that was orally administered with the *M. decumbens* methanolic root extract at a daily dose of 800 mg/kg b.wt for 28 days, and no further treatment for the following 14 days before the termination of the study, had normal weight gain and no adverse effects. This result from

the satellite group supports further the nontoxic nature of *M. decumbens* methanolic root extract in rats tested in this study (Appendix I). Since there was no or marginal reduction in body weight and relative organ weights of the treated animals at any of the tested dose levels, we conclude that the extract is nontoxic to the analyzed organs. These findings were also confirmed by the histopathological findings of the liver and kidneys after end of treatment that had normal architecture. The liver and the kidneys are target organs for toxic chemicals due to their essential functions in detoxification and excretion processes. Thus, they are considered highly useful in toxicity studies because of their sensitivity to harmful compounds and their potential to predict toxicity (Greaves, 2011).

To evaluate the probable changes in hepatic and renal functions influenced by the *M. decumbens* extract, the hematological and serum biochemical studies were done at the end of the treatment period. Liver and kidney function analysis is very important in the toxicity evaluation of plant extracts as they are both necessary for the survival of an organism (Olorunnisola *et al*., 2012). The assessment of activities of serum marker enzymes plays important role in the evaluation of plant extract for its toxicity risk. The serum enzymes considered in this study AST, ALT and ALP are valuable marker enzymes of liver cytolysis and liver cell membrane damage. AST and ALT are aminotransaminases which are good indicators of liver function and they are used as biomarkers to study probable toxicity of drugs. Normally, destruction to the liver parenchymal cells will result in an increase of both these enzymes in the blood (Wolf *et al.*, 1972). ALP is a marker enzyme for the functional integrity in plasma membranes and endoplasmic reticulum of the tissues studied. High levels of ALP are reported in liver diseases or hepatotoxicity (Brautbar & Williams, 2002). The insignificant changes in

serum ALP, AST and ALT in male and female extract treated rats in this study, at all dose levels suggest that sub-acute administration of *M. decumbens* extract did not affect the hepatocyte function in rats (Table 4.5). Additionally, the levels of total protein and albumin in serum partly indicate the status of hepatocellular and secretory functions of the liver. Significant low total serum protein in the may represent a compromised liver's synthetic ability of total protein which may suggest a liver disfunction (Fouque *et al*., 2008). There was insignificant change in serum albumin observed in both male and female treated rats compared to controls while there was significant reduction of total proteins in male and female rats treated with the highest-dose (800 mg/kg) of the extract but there were no significant changes at low dose (400 mg/kg) when compared to respective normal controls. However, the significant reduction values were within the normal range values for the animal species under the study (Appendix III).

Renal dysfunction can be measured by simultaneous measurements of urea and creatinine in serum, and their normal or reduced levels are observed when there is normal functioning of the kidney (Davis & Bredt, 1994). Higher than normal levels of serum creatinine and urea are good indicators of renal dysfunction (Whelton *et al*., 1994). Thus, the normal or significant decrease in serum creatinine concentrations with concomitant decrease in the serum urea concentration in the all the extract treated rats in this study indicates the normal functioning of the kidneys (Table 4.5). These observations were further confirmed by the histological assessment of the liver and kidney which showed normal tissue architecture (Figures 4.1 and 4.2).

The hematopoietic system is one of the most sensitive targets of toxic compounds and an important index of physiological and pathological status in both humans and animals.

The RBC and its indices, MCV, MCHC, MCH, and CH showed significant increases in the extract treated groups compared to the respective controls indicating there could be some effect on erythropoiesis, morphology, or osmotic fragility of the red blood cells, thus unfavorable (Odeyemi *et al*., 2009) (Table 4.6). White blood cells are the first line of cellular defenses that respond to infectious agents, tissue injury, or any inflammation. There was also notably no change in WBC differential count (neutrophils, lymphocytes, basophils and monocytes) which are known to rise during body defense in response to toxic environment (Agbor *et al.,* 2005). Other parameters which had significant differences from the respective controls were eosinophil, MPV, RDW, HDW, HGB and HCT. However, these significant results in hematological parameters were found to be within the normal range of variation for the animal species (Giknis & Clifford, 2008) (Appendix III) and did not exceed the toxic limits. Therefore, following 28 days of oral administration of *Maerua decumbens* methanolic root extract in male and female rats on the there was no significant toxic effect on the hematopoietic system; hence the extract is safe at the administered doses in this study.

The histopathological evaluation of the liver and kidney of normal and all *M. decumbens* root extract administered groups in this study during sub-acute toxicity testing showed no signs of toxicity (Figure 4.1 and 4.2). Nornal tissue histoarchitecture was observed after the 28 days administration of the extract. These results are in agreement to a previous study (Porwal & Maheshwari, 2017). According to Kovacic *et al*. (2005), the pathological picture observed during toxicity studies is the best indicator of the harm done to a particular organ by the chemical. The liver and kidneys are usually the first casualties of toxic substances. These histological results were concurrent with the liver

and kidney function tests as from this study, which showed normal values in all rats in 28-day sub-acute oral toxicity study. Since no signs of toxicity were observed in all the tested groups with respect to hematology, biochemistry, organ weights, body weights and histopathological examination of liver and kidney, it can be concluded that *Maerua decumbens* root methanolic extract was safer and non-toxic in rats and could be well used for pharmacological and therapeutic purposes.

In the antidiabetic study, streptozotocin (STZ) was used to induce diabetes intraperitonally in the rats and diabetes was confirmed after 5 days. The STZ-induced diabetic rats in this study had hyperglycemia after diabetes induction. The STZ-induced diabetic rat is one of the animal models that mimic the human type 1diabetes mellitus. The necrosis of pancreatic β-cells by STZ causes degranulation and reduction of insulin secretion that leads to diabetes mellitus. The alkylation nature of STZ causes *β*-cells DNA strand breaks that induce the activation of poly ADP-ribose polymerase followed by depletion of lethal nicotinamide adenine dinucleotide (Al-Awar *et al*., 2016). Also, generation of potential free radicals such as nitric oxide (NO) by intracellular metabolism of STZ aggravates the situation and precipitates further β-cells DNA strand breaks (Lenzen, 2008). The action of streptozotocin by selectively destroying the insulin secreting pancreatic cells of the islets of Langerhans results in decrease in the endogenous insulin production and thus affecting glucose utilization by the various tissues. Also, the hyperglycemia in STZ-diabetes induced rats results from decreased entry of glucose into the cells (insulin resistance) and increased production of glucose (gluconeogenesis) by the liver (ThankGod e*t al*., 2014).

The untreated diabetic rats in this study showed drastic reduction in body weight that might have resulted from the degradation of structural proteins due to unavailability of carbohydrate for the energy metabolism. Interestingly, the diabetic rats treated with *Maerua decumbens* root extract did not experience drastic weight changes compared to untreated diabetic rats, a trend that was also observed with metformin treatment (Table 4.7). Results showed that the *M. decumbens* root extract at 400 mg/kg b.wt improved the body weight in diabetic rats compared to diabetic control group after 21 days of treatment. *Maerua decumbens* extract at 400 mg/kg b.wt was more effective than at 100 mg/kg b.wt and metformin (100 mg/kg b.wt). The body weight among the rats administered with the root extract of *M. decumbens* was found to be in an increasing fashion possibly due to the reduction in glucose levels thus sparing the body fat and muscle protein which otherwise are utilized in diabetic rats or due to improved insulin which is an anabolic hormone (Mbaka *et al.,* 2012). Induction of diabetes with STZ is associated with a characteristic loss of body weight. Due to insufficient insulin, the body is prevented from getting glucose from the blood into the body's cells to use as energy. When this occurs, the body starts burning fat and muscle proteins for energy, leading to increased muscle wasting and loss of tissue proteins (Swanston *et al.,* 1990). In diabetic rats in this study, drastic reduction in body weight changes observed might be as a result of degradation or catabolism of structural proteins due to unavailability of carbohydrate for the energy metabolism.

Blood glucose measurement is one of the most relevant markers used in detecting diabetes mellitus both clinically and experimentally. In this study, the methanolic root extract of *M. decumbens* in STZ-diabetes induced rats showed blood glucose lowering

effect when administered orally for 21 days. The extract at 400 mg/kg b.wt, showed a maximum reduction of blood glucose level (62.12%) while extract at 100 mg/kg b.wt and metformin at 100mg/kg b.wt had 45.94% and 58.16% reduction in fasting blood glucose respectively when compared to the diabetic untreated control (Table 4.8). The blood glucose lowering effect of *M. decumbens* root extract may be attributed to the presence of phenols, flavonoids, tannins, alkaloids, terpenoids, sterols and cardiac glycosides that was found present in the crude extract in this study, that have been associated with hypoglycemic activity (Chauhan *et al.,* 2010). Flavonoids have insulinomimetric properties and stimulate lipogenesis and glucose transport in the adipocytes hence lowering blood sugar. Alkaloid promotes the regeneration of pancreas islets following destruction of the beta cells, hence restores the secretion of insulin, and thus corrects hyperglycemia. Terpenoids are very popular among patients with high blood pressure and diabetes because they help to reduce diastolic blood pressure and lower the sugar level in blood (Abdirahman *et al.*, 2015). Several mechanisms such as inhibition of carbohydrate metabolizing enzymes (Matsui *et al*., 2001), enhancement of glycogen regulatory enzymes expression in the liver and glucose uptake by tissues and adipocytes (Ghosh *et al*., 2004) as well as stimulation of pancreatic insulin release (Xu *et al*., 2008) have been associated with the antihyperglycemic effect of antidiabetic medicinal plants**.** Metformin is often used as a standard antidiabetic drug in STZ induced diabetes to compare the efficacy of variety of hypoglycemic compounds or plant extracts due to its easy accessibility and easy way of administration which is oral. Metformin is a biguanide which provides an effective treatment for patients with diabetes by promoting glucose uptake by GLUT4 transporters and reduces glucose production in liver. Other than its

glucose-lowering properties, metformin is also said to have an antioxidant property (Mali *et al.,* 2017).

In the current study, the effect of *M. decumbens* root extract on oxidative stress in STZ-induced diabetic rats was also examined. Administration of *M. decumbens* root extract as well as metformin reduced the diabetes-induced increase in the lipid peroxide levels, and thus reduced the susceptibility of the liver to lipid peroxidation when compared to the untreated diabetic group. Diabetics and experimental animal models of diabetes exhibit high oxidative stress due to persistent and chronic hyperglycemic state which depletes the activity of the antioxidative defense system and thus promotes *de novo* generation of free radicals (Rochette *et al*., 2014). Numerous studies have found increased lipid peroxides or reactive oxygen species (ROS) and oxidative stress (or both) in different animal models of diabetes.

Proteins, lipids, and DNA are sensitive targets of ROS (Giacco & Brownlee, 2010). STZ beta-cell cytotoxic action is thought to be mediated by the inhibition of free radical scavenger-enzymes, which enhances the production of superoxide radicals. The latter have been implicated in lipid oxidation, DNA damage, and sulfhydryl oxidation. STZ causes diabetes, and diabetes is associated with the generation of ROS, which causes oxidative damage (Kandasamy & Ashokkumar, 2013). Hyperglycemia increases the production of markers of cell damage related to free radicals, such as MDA and conjugated dienes. Lipid peroxidation may bring about protein damage and inactivation of membrane-bound enzymes either through direct attack by free radicals or through chemical modification by its end products, MDA and 4-hydroxynonenal (Dalle-Donne *et al*., 2006). MDA is a degradative product of lipid peroxidation of polyunsaturated fatty

acids (PUFA) in the cell membranes (Asuk *et al.,* 2015). In this study, the highest level of liver MDA was recorded in the diabetic untreated group, indicating that these rats were more susceptible to lipid peroxidation compared to normal rats. This result is consistent with numerous reports of increased oxidative stress in the tissues of diabetic rats (Domekouo *et al*., 2016). The decrease in MDA levels in *M. decumbens* root extract-treated rats (Figure 4.3) may be due to the presence of several phytochemical constituents including flavonoids, alkaloids, glycosides, saponins and tannins which are known natural antioxidants that possess free radical scavenging effects and rejuvenating potentials (Biswas *et al*., 2011) which have been reported to inhibit formation of lipid peroxides, owing to their ability to scavenge free radicals or which may have increased the activities of antioxidant enzymes like glutathione peroxidase in the rats and hence caused inactivation of lipid peroxidation as suggested by Afshari *et al*. (2007) or may have reduced β-cell damage or reversed impaired insulin production, its release, or function as suggested by West (2000). The results of the present study indicate therefore that the preventive effects of *M. decumbens* may be due to inhibition of lipid peroxidation as a result of its antioxidant nature. Further studies are essential to elucidate the exact mechanism of this modulatory effect.

Histopathological evaluation of liver sections obtained from STZ-diabetic rats revealed the presence of histopathological changes in the liver including; degeneration of hepatocytes and inflammation (Figure 4.4). These findings are in agreement with previous studies, which reported similar histopatholgical observations following induction of diabetes in rats using STZ (Al-Ani *et al.*, 2009). However, there was improved liver histoarchitecture observed in *M. decumbens* extract and metformin treated

diabetic rats. This appears to further be evidence that root extract or metformin treatment reversed diabetes and associated effects (Figure 4.4).

On the other hand, the photomicrographs of pancreas of STZ-induced diabetic rats showed considerable reduction and/or depleted islet of langerhans and tissue inflammations (Figure 4.5). These histopathological observations of the pancreas are in agreement with earlier reports (Jelodar Gholamali *et al*., 2005). Treatment of the diabetic rats with *Maerua decumbens* root extract ameliorated the effects of the diabetic condition as the near normal pancreatic histoarchitecture was preserved (Figure 4.5). Diabetes mellitus is a metabolic disease that affects all systems in the body, including the liver and pancreas. The primary insult to the pancreas mainly and partly liver is STZ and of course thereafter made worse by hyperglycemia and that is why all diabetic induced rats (untreated or treated) had tissue injuries especially in the pancreas. Hyperglycemia, which mainly occurs during diabetes, affects the metabolism of lipids, carbohydrates and proteins and it can lead to non-alcoholic fatty liver disease which can further progress to non-alcoholic steatohepatitis, cirrhosis and finally hepatocellular carcinomas (Mohamed *et al*., 2016). The underlying mechanism of diabetes that contributes to liver damage is the combination of increased oxidative stress and an aberrant inflammatory response; this activates the transcription of pro-apoptotic genes and damages hepatocytes. Significant involvement of pro-inflammatory cytokines including interleukin IL-1β, IL-6 and tumor necrosis factor-α exacerbates the accumulation of oxidative damage products in the liver such as MDA (Mohamed *et al*., 2016). Many factors exacerbate pancreatic tissue damage in diabetic condition (e.g. hyperglycemia/glucotoxicity, lipotoxicity, autoimmunity, inflammation, adipokines, islet amyloid, incretins and insulin resistance). Chronic

hyperglycemia may result in detrimental effects on insulin synthesis, cell survival and insulin sensitivity through multiple mechanisms: gradual loss of insulin gene expression and beta-cell specific genes; chronic endoplasmic reticulum stress and oxidative stress; changes in mitochondrial number, morphology and function; disruption in calcium homeostasis. In the presence of hyperglycemia, prolonged exposures to increased free fatty acids result in accumulation of toxic metabolites in the cells (lipotoxicity) (Cernea & Dobreanu, 2013). The mechanism of the ameliorative effect of *M. decumbens* root extract on the histopathology of liver and pancreas in STZ-induced diabetic rats may be due to the presence of phytochemicals in the root extract such as flavonoids which act as antioxidants through the direct scavenging of free radicals and thus reduce the oxidative damage of the cells caused by the diabetic state. These histopathological findings were also supported by the lipid peroxidation results (Figure 4.3) which showed a reduction in lipid peroxidation in the liver of diabetic rats treated with *Maerua decumbens* root extract.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

Phytochemical analysis of *Maerua decumbens* methanolic root extract showed presence of alkaloid, glycosides, flavonoids, phenols, saponins, steroids, tannins and terpenoids indicating its phamarcological potential. In the safety study, the results showed that the methanolic extract of roots of *Maerua decumbens* did not cause any apparent *in vivo* toxicity in rats. There were no signs of toxicity or mortality observed in rats treated with extract at doses of 2000 mg/kg b.wt in acute oral toxicity study while in sub-acute oral toxicity study, *Maerua decumbens* root extract at doses of 400 and 800 mg/kg b.wt did not also show behavioral toxicity signs or any changes in the body weights, organ weights, hepatic and renal function serum indices and hematological parameters in rats. The histological examination of the liver and kidney of extract-treated rats in the sub-acute oral toxicity study also revealed normal histoarchitecture.

The antidiabetic study indicated that *Maerua decumbens* methanolic root extract has antidiabetic activity in streptozotocin-induced diabetic rats since continued treatment with the extract lead to protection against loss of body weight; reduction of blood glucose and reduced hepatic lipid peroxidation. The root extract also showed tissue protective effect as confirmed by the improved histostology of the liver and pancreas of diabetic but extract treated rats.

6.2 RECOMMENDATIONS

Based on the outcomes of this study on the safety and antidiabetic activity of *Maerua decumbens* root extract in rats, the following recommendations are provided;

1. For traditional alternative medicine, *Maerua decumbens* root extract has a potential for the management of diabetes mellitus as it has shown;
    a. Safety when administered oral to rats.
    b. Antidiabetic efficacy in rats.
2. For future studies;
    a. Further investigations are needed to identify the lead molecules in *Maerua decumbens* root extract and to elucidate their antidiabetic mechanisms of action.
    b. Other experimental toxicity studies such as chronic oral toxicity study of *Maerua decumbens* root extract need to be undertaken to further confirm the safety of the root extract following this acute and sub-acute oral toxicity study.

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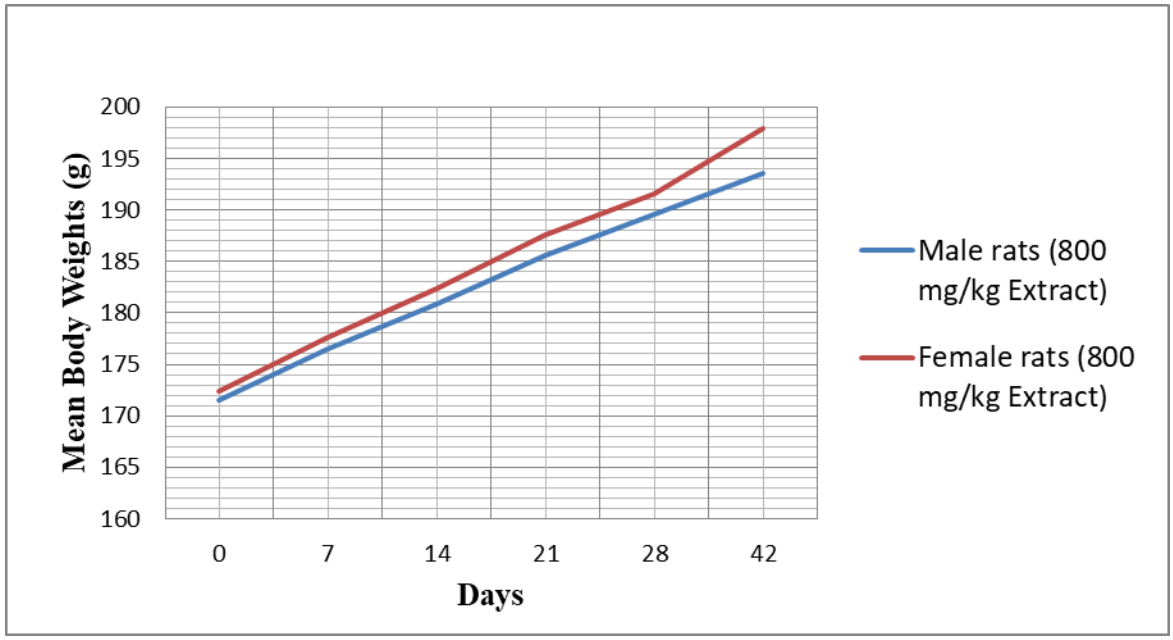
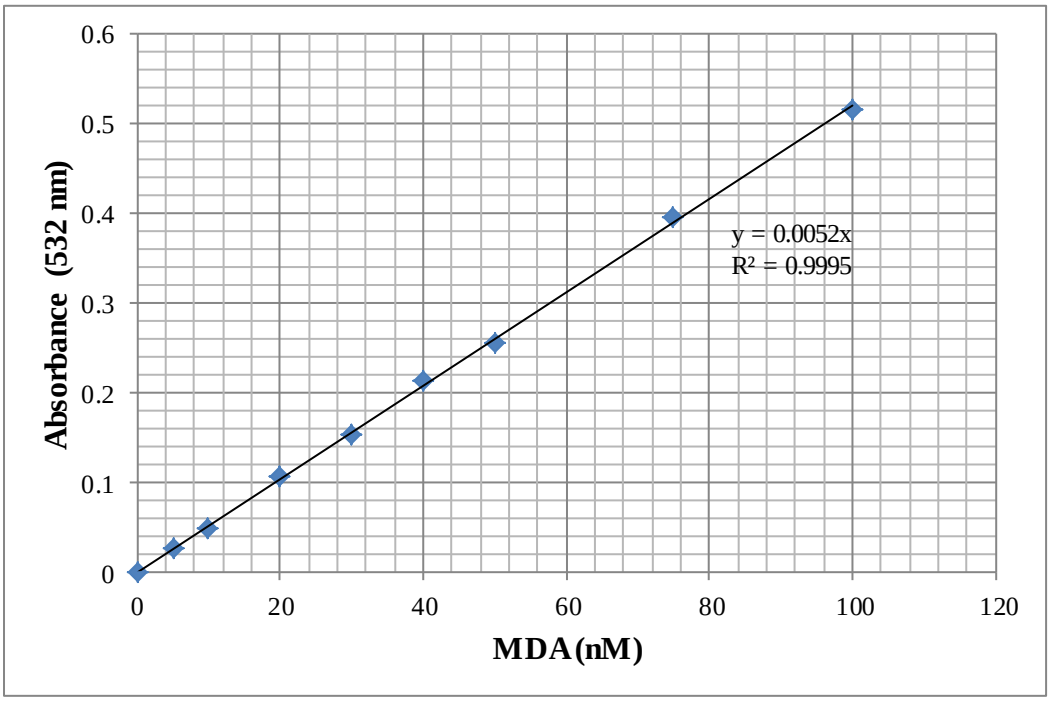
APPENDICES

Appendix I: Satellite Group Body Weights following Sub-acute Oral Toxicity Study of *Maerua decumbens* Root Extract in Rats



Appendix II: Malondialdehyde Standard Curve



Appendix III: Reference Range Values for Biochemical and Hematological Parameters for Crl: WI (Han) rats (Giknis & Clifford, 2008)

BIOCHEMICAL PARAMETERS			
Parameter	**Unit**	**Male**	**Female**
Alkaline phosphatase	U/L	62-230	26-147
Aspartate aminotransferase	U/L	74-143	65-203
Alanine aminotransferase	U/L	18-45	16-48
Total protein	g/dL	5.2-7.1	5.5-7.7
Albumin	g/dL	3.4-4.8	3.6-5.5
Urea	mg/dL	12.3-24.6	13.2-27.1
Creatinine	mg/dL	0.2-0.5	0.2-0.6
HEMATOLOGICAL PARAMETERS			
White blood cells	10^3/uL	1.96-8.25	1.13-7.49
Neutrophils	%	6.2-26.7	0.15-1.5
Lymphocytes	%	66.6-90.3	0.82-5.66
Monocytes	%	0.8-3.8	0.02-0.16
Eosinophils	%	0.2-3.5	0.01-0.15
Basophils	%	0-0.8	0-0.03
Red blood cells	10^6/uL	7.27-9.65	7.07-9.03
Hemoglobin	g/dL	13.7-17.6	13.7-16.8
Hematocrit	%	39.6-52.5	37.9-49.9
MCV	fL(um^3)	48.9-57.9	49.9 - 58.3

MCH	pg	17.1-20.4	17.8 - 20.9
MCHC	g/dL	32.9-37.5	33.2 - 37.9
CHCM	g/dL	33-37.7	33.3 - 38.1
CH	Pg	17.4-20.3	18.1 - 20.9
RDW	%	11.1-15.2	10.5 - 14.9
HDW	g/dL	2.04-3.49	1.88 - 2.81
Platelets	10^3/uL	638-1177	680 - 1200
MPV	fL(um^3)	6.2-9.4	6.2-9.8

Appendix IV: Research Ethical Clearance Letter

OFFICE OF THE DIRECTOR OF GRADUATE STUDIES AND RESEARCH

UNIVERSITY OF EASTERN AFRICA, BARATON

P. O. Box 2500-30100, Eldoret, Kenya, East Africa

March 29, 2017

Kiptisia Richard Torotich
University of Eldoret
School of Science

Dear Richard,

Re: ETHICS CLEARANCE FOR RESEARCH PROPOSAL (REC: UEAB/9/3/2017)

Your research proposal entitled ***"Elavuation of Antidiabetic Activity and Safety of Ethanolic Root Extract of Maerua decumbens in Rats"*** was discussed by the Research Ethics Committee (REC) of the University and your request for ethics clearance was granted approval.

This approval is for one year effective March 29, 2017 until March 29, 2018. For any extension beyond this time period, you will need to apply to this committee one month prior to expiry date. Note that you will need a clearance from the study site before you start gathering your data.

We wish you success in your research.

Sincerely yours,

Jackie K. Obey

University of Eastern Africa, Baraton
29 MAR 2017
P. O. Box 2500-30100, ELDORET
Research Ethics Committee

Dr. Jackie K. Obey
Chairperson, Research Ethics Committee