ANTIOXIDANT AND ANTIDIABETIC PROPERTIES OF *Mondia whitei* ROOT EXTRACT IN STREPTOZOTOCIN-INDUCED DIABETIC WISTAR RATS

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DECLARATION

Declaration by the Student

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DEDICATION

I dedicate this work to all striving to adopt and maintain a healthy eating habits and a good life style, to my family and friends.

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ABSTRACT

The rising global prevalence of diabetes mellitus (DM) and its complications presents a major health challenge and is exacerbated by the lack of a definitive cure and the side effects of existing treatments, thus, highlighting the need for safer and more sustainable anti-diabetic agents. This study investigated *Mondia whitei* root extract as an alternative therapeutic option for DM. While traditional medicine suggests that *M. whitei* possesses antioxidant and anti-diabetic properties, scientific validation is limited. This research aimed to address this gap. Qualitative phytochemicals analysis of crude root extract of *M. whitei* was done and it's *in vitro* antioxidant properties evaluated through 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity and ferric reducing antioxidant power (FRAP) assays. DM was induced in twenty-four male Wistar rats by a single intraperitoneal injection of 65 mg/Kg body weight (bwt) of streptozotocin (STZ). Animals were randomly assigned to five groups each containing six subjects; Group I (normal control, saline), Group II (diabetic control, saline), Group III (diabetic rats 200 mg/Kg bwt extract treatment), Group IV (diabetic rats 400 mg/Kg bwt extract treatment), and Group V (diabetic rats 100 mg/Kg bwt metformin treatment). Treatments were orally administered for 21 days. Fasting body weights and blood sugar levels were measured weekly. After 21 days, animals were sacrificed and their blood and liver tissue samples collected followed by serum lipid profile, liver and kidney function indices analysis. Liver malondialdehyde (MDA) levels were measured, and liver and plasma's ferric-reducing capacity were evaluated. Statistical analysis was performed using R software, with paired Student's t-test and ANOVA determining statistical significance at 95% confidence level. The qualitative phytochemical analysis of the crude *M. whitei* root extract revealed the presence of saponins, phenols, tannins, alkaloids, flavonoids, glycosides, coumarins, steroids, and terpenoids, while anthraquinones were not detected. The extract significantly scavenged DPPH radical and reduced ferric ions *in vitro*. *M. whitei* also showed significant hypoglycemic, hypolipidemic and significantly reduced serum gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). *M. whitei* treatment also significantly increased liver and blood plasma capacity to reduce ferric ions as well as protected liver tissues from lipid peroxidation as indicated by significantly reduced levels of MDA. However**,** *M. whitei* showed no significant serum urea and creatinine levels decrease. In conclusion, the phytochemical-rich *M. whitei* root extract demonstrated anti-diabetic, antioxidant, hypolipidemic and hepatoprotective effects in STZ-induced diabetic rats, highlighting its potential as a natural candidate for the management of DM and its complications.

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LIST OF ABBREVIATIONS

ADA	– American Diabetes Association
AGEs	– Advanced glycated end products
ALB	– Albumin
ALP	– Alkaline phosphatase
ALT	– Alanine transaminase
Apo A	– Apolipoprotein A
Apo B-100	– Apolipoprotein B-100
AST	– Aspartate aminotransferase
ATP	– Adenosine Triphosphate
Bwt	– Body weight
CAT	– Catalase
CVD	– cardiovascular disease
DM	– Diabetes mellitus
DPPH	– 2,2-diphenyl-1-picrylhydrazyl
EC	– Endothelial cells
EDTA	– Ethylene diamine tetra acetic acid
FBS	– Fasting Blood Sugars
FBWTS	– Fasting body weights
FFA	– Free fatty acids
FPG	– Fasting Plasma Glucose
GDM	– Gestational diabetes mellitus
GLB	– Globulins
GLP	– 1 –glucagon like peptide
H_2O_2	– Hydrogen peroxide
HbA1c	– Glycated hemoglobin
HDL	– High density lipoproteins
HLA	– Human leukocyte antigen
HSL	– Hormone sensitive lipase
IR	– Insulin resistance
ISR	– Insulin receptor

LDL – Low density lipoproteins
LPO – Lipid peroxidation
MDA – Malondialdehyde
MTP – Microsomal transfer protein
NEFA – non-esterified fatty acids
OD – Optical density
OGGT – Oral glucose tolerance test
OH - Hydroxyl radical
PKC – Protein kinase C
POD – Peroxidase
RCT – Reverse cholesterol transport
ROS – Reactive oxygen species
SdLDLs – Small dense low-density lipoproteins
SDS – Sodium dodecyl sulphate
SGLT2 – Sodium glucose cotransporter 2
SOD – Superoxide dismutase
STZ – Streptozotocin
T1DM – Type1 diabetes mellitus
T2DM – Type 2 diabetes mellitus
TBA – Thio barbituric acid
TBARS – Thio barbituric acid reactive substances
TGs – Triglycerides
TMP – Tetramethoxypropane
TNF – Tumor necrotic factor
TP - Total proteins
TRLs – Triglyceride rich lipoproteins
TZDs – Thiazolidinediones
UPR – Unfolded protein response
VEGF – Vascular endothelial growth factor
VLDL – Very low-density lipoprotein
WHO – World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background

Diabetes mellitus (DM) is a long-term metabolic disorder of elevated blood glucose levels, which is capable of damaging body organs like heart, kidneys, and nerves (Galicia-Garcia et al., 2020). Type II Diabetes Mellitus (T2DM) is the leading type of DM cases (Blahova et al., 2021; Galicia-Garcia et al., 2020a). T2DM tops the most metabolic dysfunctions and it is mostly due to impaired insulin release by pancreatic β-cells and reduced insulin response by insulin-sensitive tissues (Lu et al., 2024). Insulin is vital for glucose homeostasis and hence, the biochemical mechanisms involved in its synthesis, release, and detection are highly regulated (Galicia-Garcia et al., 2020), as any disruption in any of the mechanisms can result to a metabolic imbalance responsible for the disease onset (Galicia-Garcia et al., 2020). Majority of T2DM patients are associated with obesity or elevated body fat percentage, primarily concentrated in the abdominal region (Galicia-Garcia et al., 2020a; Lu et al., 2024).

The epidemic of DM and its impediments causes a tremendous global health threat. Half a billion people have diabetes in the world and the number might increase by 51% in 2045 (Saeedi et al., 2019). T2DM contributes highly to adult mortality, ranking on 10 top causes. By 2021, about 6.7M deaths and USD 966 billion was linked to T2DM (*IDF Diabetes Atlas 10th Edition 2021*) . By 2021, impaired glucose tolerance was estimated to be at 10.2% (551 million) globally, and projected to reach 10.8% (622 million) by 2030 and 11.6% (730 million) by 2045 (*IDF Diabetes Atlas 10th Edition 2021*). In 2015, International Diabetes Federation (IDF) reported that over 10% of people aged 20–79 years (415 million adults) had diabetes. The number may increase

by 54% by 2040, with the greatest burden being in low and middle income countries (LMICs) (Zheng et al., 2018). Prevalence of DM is at 3.3% in Kenya with a 4.5% likelihood of increase by the year 2025. In IDF Diabetes Atlas 10th edition 2021, IDF ranked Kenya in the 31st position in Africa translating to 460 victims per 10,000 population.

In modern medicine, there is still no effective treatment for DM (Rajalakshmi et al.,2009). Insulin therapy which is being commonly utilized for managing DM, has unmatched side effects such as insulin resistance, fatty liver, brain atrophy and anorexia nervosa (Rajalakshmi et al.,2009). Other drug therapies being used for management of DM involves analogues of amylin which control the rate of gastric emptying and intestinal alpha glucosidases inhibitors such as acarbose, miglitol and voglibiose which slows the rise of postprandial blood sugar (Rajalakshmi et al.,2009). These drugs are associated with several drawbacks such as hypoglycemia, liver complications, lactic acidosis and diarrhea, therefore owing to the challenges associated with the current drugs therapy there is necessity for an alternative drug with reduced adverse effects (Rajalakshmi et al.,2009).

M. whitei, a periplocoideae, is an indigenous medicinal herb in Africa which has been medically applied for generations to address various treatments (Aremu et al., 2011). The usage knowledge of this plant has been passed down using various informal literature across various ethnic groups in Africa. Some uses include treatment of fever, sexual dysfunction and bilharzia. More studies continues to highlight the local uses of *M. whitei* (Aremu et al, 2011). Across Africa, the medicinal benefits of *M. whitei* are widely deployed traditionally, with Ugandans using it to induce labor, to treat malaria in Benin and Nigeria and address impotence in Cameroon. Reports have indicated that

a portion of South Africans have utilized the power of *M. whitei* to alleviate stress in adults, jump-start appetite and treatments for fits in children (Aremu et al, 2011). Besides its use as aphrodisiac, *M. whitei* is used in treatment of poultry diseases (Oketch-Rabah, 2012). The local Luo name for *M. whitei* is "ogombo," in Kenya, while in western part specifically in Kakamega and Bungoma, it is known as "mukombero" or "mkombelo" by the Luhya people and is used to boost reproduction function and management of stomachache and rheumatism. Crushed fresh *M. whitei* roots are infused in water overnight, the resulting mixture is then filtered or decanted, and the filtrate is then drunk. The roots can also be used in stews to improve stew flavor and maintain preservation. *M. whitei* is easily accessible in Kenyan markets, where people often purchase them for chewing. One's taste buds are usually modified after chewing the root. The vendors and buyers are of both genders (Oketch-Rabah, 2012). Recently, *M. whitei* is used to treat diabetes and hypertension, as a flavoring agent, and as a galactagogic (Oketch-Rabah, 2012). In addition*, M. whitei* is keenly used in Kenya for treating heart diseases, Asthma, stomach-related worms and skin diseases (Aremu et al., 2011). Sequelae of diabetes pose a significant threat to human health besides invoking financial burden and despite this being the case, there exist no cure for DM and even the existing management treatments have various side effects. This study therefore aimed at exploring the antioxidant and antidiabetic properties of *M. whitei* root extract*,* which has the potency to provide an alternative medicine to address diabetes and its complications with reduced side effects and ultimately aid in easing the global health threat and financial burden being implicated by diabetes mellitus.

1.2 Statement of the problem

The epidemic of DM and the aftereffects pose a major global health threat and financial burden. The number of diabetic patients is on rise implicating more global threat and financial burden in the future. This figure is expected to double with LMICs bearing most impact due to increased rate of industrialization (Emordi et al., 2016,). There are over half a billion cases of diabetes and an upward trend is expected by 2045, with 41% new cases recorded (Saeedi et al., 2019). In 2024, diabetes caused I trillion USD health expenditure globally (IDF Diabetes Atlas 11th Edition 2025). Kenya's diabetes prevalence was reported at 3.1% with a possible rise to 1.8 million in 2050 (IDF Diabetes Atlas 11th Edition 2025). Diabetes was responsible for 3.4 million deaths, which translates to 1 in every 9 seconds in 2024 (IDF Diabetes Atlas 11th Edition 2025).According to IDF Atlas 10th Edition 2021, Kenya was ranked at 31st position regionally. There is no dependable therapy available to cure T2DM even at the verge of modern medicine development. Insulin therapy has side effects despite being widely utilized. Other approaches being used to regulate hyperglycemia include the use of amylin analogues, inhibitors of intestinal alpha glucosidases, sulphonylureas and metformin (Rajalakshmi et al., 2009). Insulin therapy is linked to drug resistance, toxicity and other side effects. Sulfonylureas, for instance, lose its effectiveness after treatment in 4 out of 10 patients, besides glucose-lowering drugs are allegedly not able to control hyperlipidemia (Kooti et al., 2016). Moreover, they cause hypoglycemia, liver problems, diarrhea and lactic acidosis (Rajalakshmi et al. 2009). These side effects calls for the need for a safer agents with minimized effects. (Rajalakshmi et al. 2009). Medicinal plants have reduced side effects, and they are more affordable. The high level of treatment failures, unpleasant side effects and enormous cost associated with diabetic

therapy have generated an urgent need and desire for alternative treatments (Emordi et al., 2016,). This study aimed at getting an alternative agent for treating diabetes mellitus from *M. whitei* root extract with reduced side effect and ultimately help ease the financial burden and global health implicated by diabetes.

1.3 Justification of the study

Diabetes related consequences pose a major global health threat and has contributed tremendously to the burden of mortality and disability worldwide besides the financial burden. Due to the adverse side effects associated with conventional drugs, a safer agent with minimal side effects is appropriate for extended periods (Rajalakshmi et al.,2009). Studies have validated that various phytochemicals possess anti-hyperglycemic potentials capable of being utilized in diabetic and metabolic complications management hence mitigating conventional drugs undesirable effects, however, a significant number of plants and their bioactive ingredients are still understudied (Alam et al., 2022,).

M. whitei was used in this study based on the following reasons;

- ✓ Pereira and group did a Diabetes-Data Base virtual screening of African medicinal for potential anti-diabetic compounds. *M. whitei* was one of the plants screened and they found out that it contained 5-chloropropacin and 7-hydroxy -4-8-dimethoxypropacin compounds (Pereira et al., 2019).
- ✓ Studies have reported *Mondia whitei* to contain 2-hydroxy-4-methoxy benzaldehyde, a compound whose derivative might have some antidiabetics and antioxidants properties (Kannabiran & Gayathri, 2009).
- ✓ According to Esikuri et al. (2005*) M. whitei* has been utilized in diabetes and hypertension treatment.

An alternative intervention with minimized side effects is ideal owing to global health threat emanating from DM and its complications. *M. whitei* has medicinal value and has numerous bioactive molecules. Several compounds of *M. whitei* or their derivatives have been confirmed to contain some antidiabetic properties, hence *M. whitei* has the potential to meet the existing gap and this research focused at exploring that antidiabetic potential as it had not been explored.

This study used male Wistar rats which are commonly used in study of diabetes. The pancreatic beta cells of the male rats are more prone to STZ cytotoxicity compared to the female rats (Furman, 2015). For therapeutic-drug experiments as well as foundational studies, Chemically-induced streptozotocin model (STZ) is widely used as it generates a rapid onset of hyperglycemia within a few days after injection (Naderi et al., 2019). STZ induces pancreatic islet β-cell destruction hence appropriate to produce diabetic models (Furman, 2015).

Root crude extract of *M. whitei* was used since the root is the most consumed and commercialized part of *M. whitei*. Crude extract was utilized as a foundational element, targeting to be utilized as a baseline for subsequent studies. Also, because people consume the whole root of white ginger. Aqueous solution, methanol and hexane were selected for extraction based on their success as reported by (Watcho et al., 2007).

1.4 Study objectives

1.4.1 General objective

To investigate the antioxidant and antidiabetic properties of *M. whitei* crude root extract in streptozotocin-induced diabetic Wistar albino male rats.

1.4.2 Specific objectives

The specific objectives of this study were.

1. To qualitatively determine the phytochemical composition of *M. whitei* crude root extract.

2. To determine the *in vitro* antioxidant properties of the *M. whitei* crude root extract.

3. To determine the effects of crude *M. whitei* root extract on FBS and body weights in streptozotocin-induced diabetic Wistar rats.

4. To determine the effects of crude *M. whitei* root extract on serum liver and kidney function indices, lipid profile, liver lipid peroxidation and *in vivo* antioxidants capacity in streptozotocin induced diabetic Wistar rats.

1.5 Hypothesis

1. There are no phytochemicals in *M. whitei* crude root extract.

2. There exist no *in vitro* antioxidant properties of *M. whitei* crude root extract.

3. *M. whitei* root extract has no effect on fasting blood glucose and body weights in streptozotocin-induced diabetic Wistar rats.

4. Crude *M. whitei* root extract has no effect on serum liver and kidney function indices, lipid profile, liver lipid peroxidation, and antioxidant capacity in streptozotocin-induced diabetic Wistar rat.

1.6 Overall significance of the study

This research provides a scientific basis for the utilization of *M. whitei* root extracts as a prospective antioxidant and herbal remedy for diabetes. The knowledge derived from the study may inspire further exploration of other antidiabetic and antioxidant properties of *M. whitei* root extract.

CHAPTER TWO

LITERATURE REVIEW

2.1 Diabetes mellitus

Diabetes mellitus (DM) is a long term metabolic disease caused by insufficient insulin secretion and/or resistance of cells to insulin, which as a consequence blood sugar levels get elevated, uncontrolled chronic hyperglycemia may result to tissue damage and dysfunction, and failure of the eyes, heart, blood vessels, nerves, and kidneys (Mbanya & Ramiaya 2006; Solis-Herrera et al., 2021). Risk factors associated with diabetes include genetic disorders, diseases that cause harm to the pancreas, excess secretion of hormones such as growth hormones and glucocorticoids, drugs, or chemicals (Solis-Herrera et al.,2021).

2.2 Types of diabetes mellitus

The most common types include T1DM, T2DM, and GDM.

2.2.1 Type 1 diabetes mellitus

T1DM usually onset early and is often diagnosed in kids and young adults; hence also called juvenile onset diabetes(Li et al., 2017). T1DM manifests as a consequence of pancreatic β cells destruction through autoimmune reaction of CD4+, CD8+, T cells and macrophages invading the islets cells, resulting to absolute deficiency of insulin (American Diabetes Association, 2021; Gillespie, 2006). As a consequence of β-cells destruction in patients with T1DM there blood glucose regulation lose, and this can potentially lead to acute conditions such as, severe hypoglycemia and ketoacidosis (Bluestone et al., 2010) . Even in the existence of insulin replacement therapy secondary complications such as cardiovascular disease, vision impairment and renal failure can still come in the picture (Bluestone et al., 2010).

2.2.2 Type 2 diabetes mellitus (T2DM)

T2DM manifests as a consequence of accumulative loss of adequate beta-cell causing insulin secretion impairment and is often accompanied by insulin resistance (American Diabetes Association, 2021). T2DM ranks among prominent metabolic disorder as it accounts for over 90% of diabetes mellitus cases, this metabolic disorder develop as a consequence of defective insulin secretion by pancreatic β-cells and the inability of insulin-sensitive tissues to respond optimally to insulin (Galicia-Garcia et al., 2020). Pancreatic beta cells dysfunction, as indicated by recent evidence is a consequence of a network of interactions between the environment and different molecular pathway implicating the cellular biology (Galicia-Garcia et al., 2020). Contrary to insulin insensitivity which is an early phenomenon partly related to obesity, functionality pancreas β-cell declines gradually over time before the onset of clinical hyperglycemia (Stumvoll et al., 2005). Mechanisms such as increased non-esterified fatty acids, inflammatory cytokines, adipokines, and mitochondrial dysfunction for insulin resistance have been proposed, and in the case of β-cell dysfunction, glucotoxicity, lipotoxicity, and amyloid formation have been proposed (Stumvoll et al., 2005). In instances of excess nutritive state as such found in obesity, hyperglycemia, and hyperlipidemia, chronic inflammation and IR are favored, and in this conditions beta cells get subjected to toxic pressures such as inflammation stress, stress from amyloid accumulation, and ROS stress which can ultimately lead to loss islets cells integrity (Galicia-Garcia et al., 2020). Glucotoxicity, lipotoxicity, and glucolipotoxicity as found in obesity bring about metabolic and oxidative stress that contribute to beta cell damage (Galicia-Garcia et al., 2020). Moreover, the disease has a strong genetic component, but only a handful of genes have been identified so far: genes for calpain 10, potassium

inward-rectifier 6·2, peroxisome proliferator-activated receptor, insulin receptor substrate-1, and others, management includes not only diet and exercise, but also combinations of antihyperglycemic drug treatment with lipid-lowering, antihypertensive, and anti- platelet therapy (Stumvoll et al., 2005)

β-cell impairment is a critical component in the progression of type 2 diabetes (Stumvoll et al., 2005). When insulin action decreases the system usually compensates by increasing β-cell function, at the same time, concentrations of blood glucose at fasting and 2 h after glucose load will increase mildly, though this increase may be small, over time it becomes damaging because of glucose toxicity, and in itself a cause for β-cell dysfunction, therefore, even with unlimited β-cell reserve, insulin resistance paves the way for hyperglycemia and T2DM (Stumvoll et al., 2005)

Insulin resistance is in effect when the biological impact of insulin is significantly low in both glucose disposal uptake in skeletal muscle and inhibition of glucose production in the liver (Stumvoll et al., 2005). Excess secretion of glucocorticoids and catecholamine maybe responsible of inducing IR as they promote lipolysis, glycogenolysis, and muscle catabolism (Galicia-Garcia et al., 2020b). Gluconeogenesis is normally elevated in type 2 diabetes patients, since this increase occurs simultaneously with hyperinsulinemia, at least in the early and intermediate disease stages, hepatic insulin resistance is the driving force of hyperglycemia of T2DM (Stumvoll et al., 2005). Insulin resistance is strongly linked to obesity and inactive life style, and some mechanisms supporting this interaction have been put to light, some hormones, cytokines, and metabolic fuels, such as non-esterified (free) fatty acids (NEFA) originate in the adipose tissues and regulate insulin action (Stumvoll et al., 2005). Increased storage of triglyceride, especially in visceral or deep subcutaneous

adipose depots, leads to large adipocytes which are resistant to the ability of insulin to suppress lipolysis and in turn results in increased release and circulating levels of NEFA and glycerol, both of which aggravate insulin resistance in skeletal muscle and liver (Stumvoll et al., 2005)

2.2.3 Gestational diabetes mellitus (GDM)

GDM Occurs during pregnancy, and mostly manifest during the third trimester of the pregnancy, women who get GDM have a great risk of developing T2DM later on (Solis-Herrera et al.,2021). GDM is a threatening pregnancy complexity and is associated with approximately 16.5% of pregnancies worldwide, in which women with no prior diabetic conditions develop chronic high blood glucose during gestation as a consequence of impaired glucose regulation due to impaired pancreatic β-cell function building on chronic insulin resistance, the key pathophysiological features of insulin resistance and defective insulin secretion mirror those observed in T2DM (Johns et al., 2018, Plows et al., 2018).

2.3 Epidemiology of T2DM

Epidemiological data shows shocking values that predict a worrisome future for T2DM. According to WHO (2022), cases and the prevalence of diabetes have been rising steadily over the past few decades. The widespread of DM and its complexities pose a major global health threat, according to IDF in adults of age 20-79 years, 1 out of 11 had DM worldwide in 2015, and there is prediction of continuous rise, with largest increases being expected in regions undergoing economic growth from lower to middle-income brackets (Zheng et al., 2018a). Prevalence of DM in 2017 was 425 million, by 2030 a 20% rise is estimated in the figures of adults with diabetes mellitus in developed countries and a 69% rise in developing countries, besides it is estimated

to elevate to 629 million cases by 2045 (Forouhi & Wareham, 2019; Zheng et al., 2018a). According to the International Diabetes Federation (IDF), in 2019, diabetes caused 4.2 million deaths, and 463 million adults aged between 20- and 79-years old were living with diabetes, a number that will likely rise up to 700 million by 2045. Diabetes was the underlying cause of at least 720 billion USD in health expenditure in 2019. In 2024, 589 million adults (20-79 years) were living with diabetes globally (1 in 9), and this number is expected to rise to 853 million by 2050 (IDF Diabetes Atlas 11th Edition 2025).In the same year of 2024, diabetes caused I trillion USD health expenditure globally (IDF Diabetes Atlas 11th Edition 2025).

Additionally, the factual disease burden of T2DM is likely an underrepresentation as 1 in 3 diabetic people were underdiagnosed, equivalent to 232 million people. The greatest number of people suffering from diabetes are aged between 40 and 59 years old. Prevalence and incidence of T2DM vary according to geographical region, with more than 80% of patients living in low-to-middle-income countries, which poses surplus challenges in effective treatment (Galicia-Garcia et al., 2020a).

T2DM and its complications have contributed tremendously to the burden of mortality and disability worldwide and is the ninth (9^{th}) major cause of reduced life expectancy, in 2010, it was estimated that diabetes mellitus caused 3.96 million deaths in adults aged 20–79 years during that year (6.8% of global mortality), an estimate which rose to 5.0 million deaths during 2015 in an IDF report, which is equivalent to one death per every six seconds (Zheng et al., 2018b). In 2024, diabetes was responsible for 3.4 million deaths, which translates to 1 in every 9 seconds (IDF Diabetes Atlas 11th Edition 2025).

In Kenya according to WHO, diabetes prevalence is at 3.3 % and projected to rise to 4.5% by 2025. IDF ranked Kenya as the 31st African country in terms of diabetes with prevalence of 460 cases per 10,000 populations. Kenya's diabetes prevalence was reported at 3.1% with a possible rise to 1.8 million in 2050 (IDF Diabetes Atlas 11th Edition 2025).

2.4 Etiology and risk factors of T2DM

A good number of factors are associated with T2DM, these factors includes lifestyle choices (smoking, sedentary lifestyle, alcohol consumption), eating habits, genetic predisposition, among others as summarized in figures 2.1 and 2.2 below.

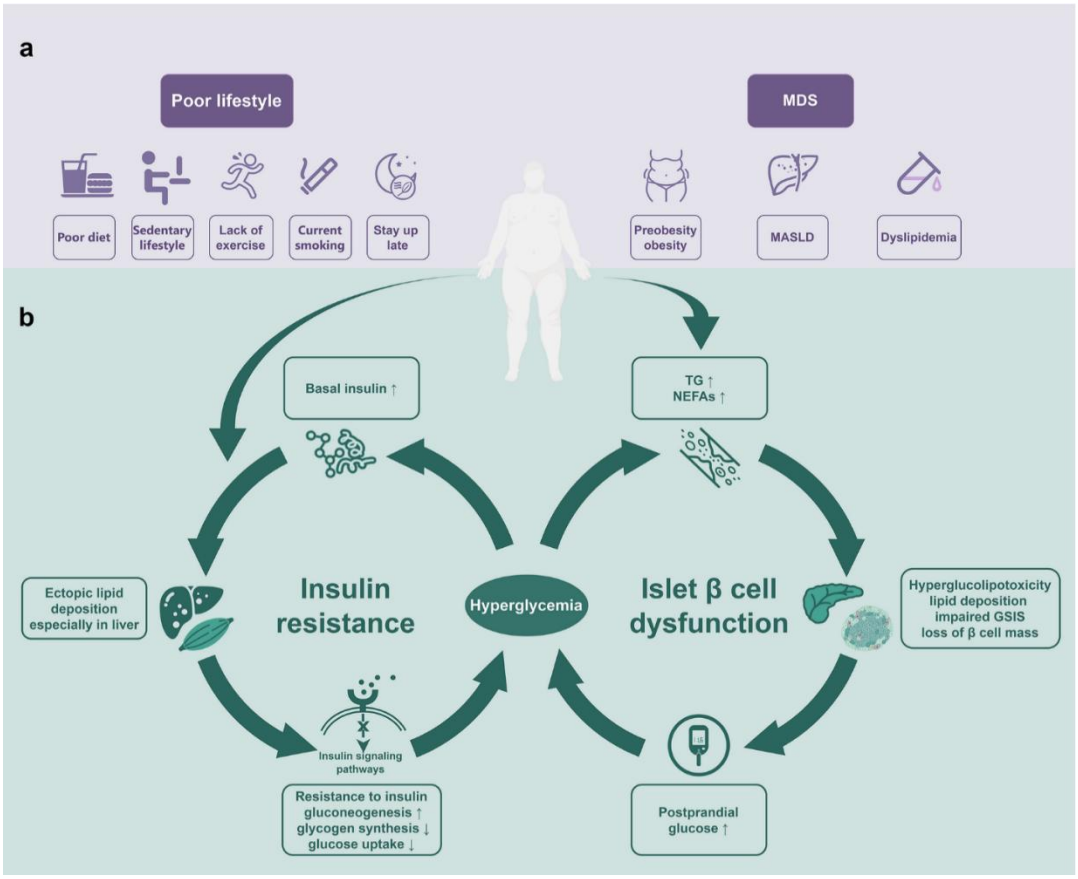

Figure 0.1; A summation of the contributing factors and underlying mechanism of T2DM (Lu et al., 2024)

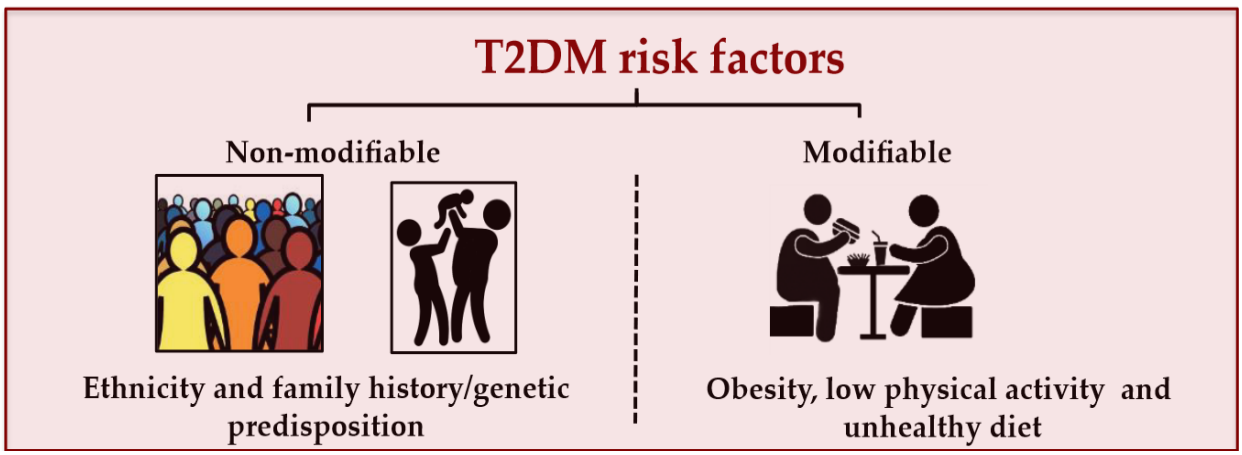

Figure 0.2; T2DM risk factors. (Source: Galicia-Garcia et al., 2020).

2.4.1 Psychological stress

Cortisol plays a critical biological functions, many of which relates to diabetes mellitus, for instance, glucocorticoid hormones are called so due to their influence on glucose levels regulation (Hackett & Steptoe, 2017). During stress, cortisol stimulates energy stores mobilization , which as a consequence lead to glucose and lipids release into blood circulation, additionally cortisol release suppresses inflammation responses and enhances the cardiovascular system (Hackett & Steptoe, 2017). Cardiovascular system enhancement lead to increases in blood pressure by working through sympathetic system, which innervates various tissues and works alongside adrenaline release from the adrenal medulla to elevate heart rate and blood pressure, reduce heart rate variability and promote energy mobilization and trigger the release of pro-inflammatory cytokines1 (Hackett & Steptoe, 2017)

In relation to T2DM, excessive availability of glucose or lipids due to cellular energy needs contribute to a form of metabolic condition that can contribute to insulin resistance and gaining of weight, long durations of elevated levels of glucose can destroy mitochondria and mitochondrial DNA, which as a consequence can enhance inflammation and shortening of telomere (Hackett & Steptoe, 2017)

Corticosterone (glucocorticoid) is involved in rodent physiological response to stress; prolonged corticosterone administration via drinking water induces hyperglycemia, insulin resistance and dyslipidemia in rodents (Hackett & Steptoe, 2017). Conversely, adrenal gland removal of the in rodents enhances brain insulin sensitivity, suggesting that the absence of glucocorticoids in circulation improves insulin responsiveness (Hackett & Steptoe, 2017). Similarly, patients with chronic cortisol excess, such as those with Cushing syndrome, or undergoing glucocorticoids therapy often exhibit

increased susceptibility to hyperglycemia and develop diabetes mellitus (Hackett & Steptoe, 2017).

2.4.2 Genetic factors

Substantial evidence from twin and family studies has suggested a genetic basis of T2DM; most of these genetic factors contribute to T2DM by effecting on insulin production, and a small portion by reducing action of insulin (Zheng et al., 2018c, p. 92). Approximately 75 T2DM susceptibility loci have been identified (Wu et al., 2014). They include KCNJ11 (potassium inwardly rectifying channel, subfamily J, member 11), TCF7L2 (transcription factor 7–like 2,), IRS1 (insulin receptor substrate 1), MTNR1B (melatonin-receptor gene), PPARG2 (peroxisome proliferator-activated receptor gamma 2), IGF2BP2 (insulin-like growth factor two binding protein 2), CDKN2A (cyclin-dependent kinase inhibitor 2A), HHEX (haematopoietically expressed homeobox), FTO (fat mass and obesity associated) gene and low IL-10 production capacity (Wu et al., 2014). Several genes interaction with the environment lead to diabetes induction (Kahn et al., 2006,). The P12A polymorphism in PPARγ gene variant is mostly associated with insulin sensitivity, some genes related to β-cell impairment include hepatocyte nuclear factor-4α and 1α (Kahn et al., 2006,)

2.4.3 Life style/ environmental factors

Various lifestyle factors for instance sedentary lifestyle, physical inactiveness, smoking, and alcohol consumption have been linked to the development of T2DM (Zheng et al., 2018c). Although genetic predisposition partly determines individual susceptibility to T2DM, a sedentary lifestyle and an unhealthy diet are important drivers of the current global epidemic; early developmental factors (such as intrauterine exposures) also contribute to T2DM susceptibility later in life (Zheng et al., 2018c).

Moreover, a high level of exposure to second-hand smoke has been associated with can increased risk of T2DM, smokers are more likely to have central fat accumulation than non-smokers, and smoking is known to stimulate resistance of insulin and compensatory insulin-secretion responses, which could explain the elevated risk of smokers getting T2DM (Zheng et al., 2018).

High energy Western-style diet in combination with a sedentary lifestyle, is a leading contributor to T2DM and is also considered a key driver of obesity epidemic, which is strongly linked to the increasing rate of T2D (Kolb & Martin, 2017). A closer examination on elevated body mass index (BMI) appears to have lesser impact on T2DM risk compared to increased visceral obesity and/or ectopic fat, particularly in the liver (Kolb & Martin, 2017).

Epidemiological studies indicate that intense to moderate physical activity lowers diabetes risk by nearly 30% while on the contrary all types of leisure time activities and occupational physical activity were shown to have an inverse association with diabetes risk(Kolb & Martin, 2017). For instance, it was discovered that every hour spent watching television raised the risk of diabetes development by over 3.4% in 3.2 years, so elongated duration of sedentary behavior may quadruple danger of diabetes (Kolb & Martin, 2017). Passive or active smoking of cigarettes has been related to a higher risk of T2DM compared to non-smoking individuals; heavy smokers have especially great risk when compared to lesser smokers or someone who used to smoke (Kolb & Martin, 2017).

Since the fundamental cause of T2D is inadequate insulin production, environmental and lifestyle elements have to either directly or indirectly induce harm to β-cells. (Kolb & Martin, 2017). Additional diabetes risk factors influence different sites with most

worsening inflammation and subsequent insulin resistance; few are likely to directly alter function of β-cell, with possible exceptions being raised levels of nutrients and their metabolites in circulation (Kolb & Martin, 2017).

2.4.4 Obesity

Extensive studies have demonstrated that obesity is the primary risk factor for T2DM playing an important role in the onset of the insulin resistance and disease progression (Wu et al., 2014a; Zheng et al., 2018c). Approximately 90% of diabetic patients develop T2DM, largely due to excess body weight (Wu et al., 2014a; Zheng et al., 2018c). Excess adiposity, assessed by a high BMI, is the most important risk factor for T2DM and is linked to many metabolic abnormalities that result in insulin resistance, 61% T2DM cases could be attributed to overweight (defined as a BMI ≥25 kg/m2), furthermore, abdominal obesity assessed by waist circumference or waist-hip ratio predicts T2DM risk independent of BM (Wu et al., 2014a; Zheng et al., 2018c) The exact nature of the mechanism, or mechanisms, promoting type 2 diabetes in susceptible obese individuals has not yet been identified, obesity is linked to elevated insulin resistance which is a predisposing factor for T2DM (Maggio & Pi-Sunyer, 2003).

Current theories indicate that T2DM advances when pancreatic beta-cell output can no longer satisfy the demands imposed by increased insulin resistance, cytokine tumor necrosis factors, whose expression in and secretion from adipose tissue is elevated in obesity, may contribute to insulin resistance, Adiponectin is another secretory product from adipose tissue that may be involved in the onset of T2DM in susceptible obese individuals (Maggio & Pi-Sunyer, 2003,). A mechanistic explanation suggests that the physical change of the receptor configuration caused by stretching the cell surface of

enlarged adipocytes reduces the efficiency of glucose transport; a biochemical explanation is that over-nutrition cause's oxidative stress, which results in oxidation induced inactivation of the glucose transporter GLUT4, therefore causing individuals with excessive fat storage to have higher insulin levels and thus said to be insulin resistance, which is the cause of T2DM; this form of insulin resistance is acquired in proportion of accumulated fat (Malone & Hansen, 2019).

Obesity is the most significant factor leading to development of metabolic diseases (Kahn et al., 2006). Adipose tissue regulates metabolism by releasing hormones (leptin and adiponectin), non-esterified fatty acids (NEFAs), glycerol, and pro inflammatory cytokines , in obese cases most of these products are overproduced (Kahn et al., 2006). Furthermore, increased release of monocyte chemoattractant protein-1 (MCP-1), interleukin-6, tumour necrosis factor-α, and other macrophage products may be a factor in the development of insulin resistance. (Kahn et al., 2006). NEFA synthesis may be the most significant component in developing insulin sensitivity, with their higher levels being proven in obesity and T2DM, in humans insulin resistance onsets within hours of an acute spike of plasma NEFA (Kahn et al., 2006, p. 840).

2.4.5 Diet

A low-fiber diet with a high glycemic index is positively associated with an elevated risk of T2DM, while specific dietary fatty acids can influence insulin resistance and diabetes risk to varying degrees. (Wu et al., 2014). Independent of BMI, saturated fat intake is associated with a higher likelihood of developing T2DM besides frequent consumption of processed meat, but not other types of meat (Wu et al., 2014). Soft drinks have been closely related to an increased risk of T2DM and metabolic syndrome, mostly due to their direct impact on BMI. (Wu et al., 2014).

Increased caloric intake and fat consumption, coupled with reduced physical activity, contribute to over nutrition, excessive nutrient storage, and obesity (Kahn et al., 2006). Chronic intake of high dietary fat is linked not only to obesity but as well as to decreased insulin secretion (Kahn et al., 2006). Additionally, balance shift of dietary carbohydrates and fats can affect both insulin secretion and sensitivity within just three days, even before obesity matures to be a contributing factor to diabetes (Kahn et al., 2006) . Another proposed environmental mechanism occurs in utero and/or during the early postnatal period, where insufficient nutrition interferes with metabolism, leading to cells adaptations that enhance storage of nutrients (Kahn et al., 2006). Negative genetic factors interactions with this environmental changes ultimately elevates the risk of obesity and T2DM (Kahn et al., 2006).

Considering the diverse dietary regimens across various regions, it is then not a surprise that prospective epidemiological studies show some discrepancies in the relation between food groups and T2DM occurrence (Kolb & Martin, 2017). However, foods based on plants are linked to a lower T2D risk compared to meat, and at the same time low-energy-density foods have extremely reduced risk of T2DM than high-energy-density foods (Kolb & Martin, 2017). Sugar-sweetened drinks and grains that are refined contribute immensely to obesity and an increased risk of diabetes (Kolb & Martin, 2017).

2.5 Pathophysiology of T2DM

Pathogenesis of T2DM has been strongly linked to environmental and genes (Östenson, 2001). The progression of T2DM has been linked to two major factors; β-cell dysfunction, resulting in impaired insulin production, and insulin resistance (IR) which impairs insulin action and consequently limits the body's potential to regulate glucose

consequently promoting the liver to produce glucose and inhibits uptake of glucose in the muscle tissues, adipose tissue and liver (Galicia-Garcia et al., 2020, Ozougwu, 2013). This malfunctioning feedback loops between the insulin action and insulin production results to hyperglycemia which in return promotes development of T2DM (Galicia-Garcia et al., 2020).

2.5.1 Mechanisms for β-Cell dysfunction

Dysfunction of beta cells is critical to the development of T2DM (Stumvoll et al., 2005). For insulin levels to be properly regulated to meet metabolic demand, appropriate structural integrity of islet must be conserved to enable β-cells to respond appropriately to metabolic needs (Galicia-Garcia et al., 2020). Under pathogenic conditions, islet integrity is interfered with, impairing optimal cell-to-cell communication within pancreatic islets, contributing to poor glucagon and insulin secretion demand response hence bringing about hyperglycemia (Galicia-Garcia et al., 2020). When insulin action decreases (as it happens with increasing diabetes) the body compensates by increasing beta cell function to produce more insulin, however at the same time concentration of glucose continues to build up as in the case of increasing obesity even if mildly but eventually become damaging due to glucose toxicity and in itself cause beta cells dysfunction (Stumvoll et al., 2005).

Impaired insulin secretion typically worsens over time, with its progression influencing glucose toxicity and lipotoxicity, if left untreated, these factors have been shown in animal studies to reduce pancreatic cell mass. (Scheen,2003). β-cell dysfunction was previously linked to β-cell death; however, recent studies suggests that in T2DM, β-cell dysfunction may result from a complex interplay between environmental factors and various molecular pathways (Galicia-Garcia et al.,2020). An exaggerated nutritional

state, as in the case of obesity, along with hyperlipidemia and hyperglycemia, often promotes chronic inflammation and insulin resistance (Galicia-Garcia et al.,2020). Under these conditions, β-cells are exposed to various toxic pressures, including inflammatory stress, endoplasmic reticulum (ER) stress, and oxidative stress which may ultimately compromise islet integrity (Galicia-Garcia et al.,2020). Hyperglycemia and excess of FFAs contribute to β-cell dysfunction by triggering ER stress through the activation of apoptotic unfolded protein response (UPR) pathways, in obesity, lipotoxicity, glucotoxicity, and glucolipotoxicity induce metabolic and oxidative stress, ultimately leading to β-cell damage (Galicia-Garcia et al.,2020). Stress caused by elevated levels of saturated FFAs can activate the unfolded protein response (UPR) through multiple mechanisms, including inhibition of the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA), which regulates ER Ca^{2+} mobilization, activation of IP_3 receptors, or direct disruption of ER homeostasis (Galicia-Garcia et al.,2020). In addition to sustained high glucose levels enhancing proinsulin biosynthesis, they also increase islet amyloid polypeptide (IAPP) production in β-cells, resulting in the accumulation of misfolded insulin and IAPP (Galicia-Garcia et al.,2020). This process further amplifies oxidative stress by promoting the generation of reactive oxygen species (ROS) through oxidative protein folding (Galicia-Garcia et al.,2020). This, in turn, disrupts physiological ER Ca^{2+} mobilization, promoting proapoptotic signaling, proinsulin mRNA degradation, and the release of interleukin (IL)-1β, the release of IL-1β recruits macrophages, further exacerbating local islet inflammation (Galicia-Garcia et al., 2020)

Defects in the synthesis of insulin precursors, insulin itself, or the secretion mechanism can result in insulin secretory dysfunction, a key contributor to β-cell failure and a

fundamental aspect of T2DM (Galicia-Garcia et al., 2020). For example, reduced expression of the GLUT2 glucose transporter can impair downstream signaling pathways, as shown in Figure 2.3, on top of aforementioned improper folding of proinsulin is frequently associated with inadequate insulin production and the development of diabetes (Galicia-Garcia et al., 2020).

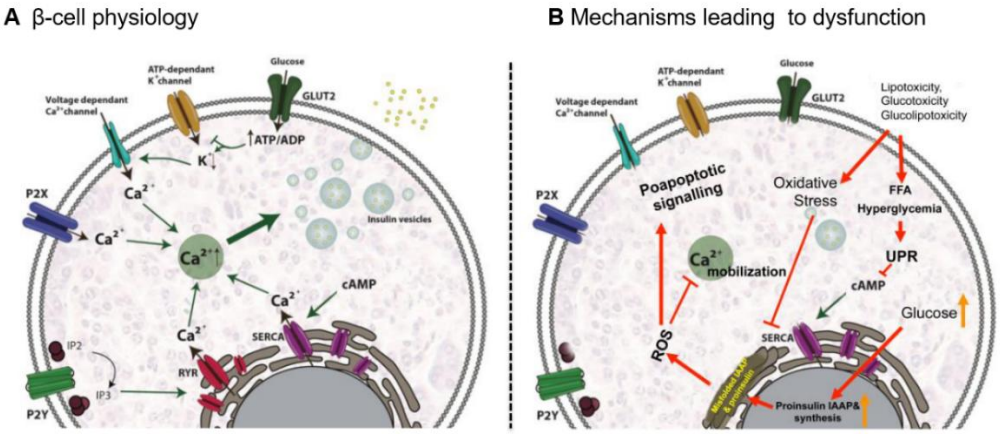

Figure 0.3; Insulin Secretion Pathways in β-Cells Under Normal Conditions (A) and Dysfunctional Mechanisms (B) (Galicia-Garcia et al., 2020c).

2.5.2 Insulin resistance

Insulin resistance (IR) is characterized by a reduced metabolic response of insulin-sensitive cells to insulin and can be classified into three main categories namely decreased insulin secretion by β-cells, the presence of insulin antagonists in the plasma, either due to counter-regulatory hormones or non-hormonal factors that disrupt insulin receptors or signaling, and impaired insulin responsiveness in target tissues (Galicia-Garcia et al., 2020).

Genetic factors influencing insulin resistance extend beyond polymorphisms in the insulin receptor and insulin receptor substrate (IRS)-1 genes, which directly impact insulin signaling. They also include variations in thrifty genes, such as the β3-adrenergic receptor gene and uncoupling protein (UCP) gene, both linked to visceral obesity and the promotion of insulin resistance. Additionally, glucolipotoxicity and inflammatory mediators play crucial roles in disrupting insulin secretion and impairing insulin signaling pathways (Kaku, 2010). Mutations that decrease the expression of the insulin receptor or GLUT4, along with defects in either upstream or downstream signaling pathways, can impair glucose uptake in muscle tissue, ultimately leading to a hyperglycemic state (Galicia-Garcia et al., 2020).

The activation of INSR tyrosine kinase is crucial for insulin's role in glucose metabolism as insulin binding to the α-subunit of the INSR, triggers phosphorylation of the β-subunit at multiple tyrosine residues which in turn initiates insulin-mediated signaling (Galicia-Garcia et al., 2020). Mutations in key phosphorylation sites can alter INSR tyrosine kinase activity, thereby inhibiting insulin action in skeletal muscle, additionally, mutations in essential downstream signaling proteins, such as IRS-1, IRS-2, or phosphoinositide 3-kinase (PI3K), can further compromise insulin function in muscle tissue (Galicia-Garcia et al., 2020).

Excess abdominal fat is linked to elevated NEFA release, which can diminish insulin sensitivity in both the liver and muscle tissues (Scheen, 2003). Chronic inflammation, linked to excess abdominal fat, plays a key role in IR and T2DM (Galicia-Garcia et al., 2020). Obesity leads to increased immune cell infiltration and the secretion of proinflammatory molecules within intramyocellular and perimuscular adipose tissue,

resulting in skeletal muscle inflammation which contributes to myocyte dysfunction, impairs metabolism, and exacerbates IR through paracrine signaling (Galicia-Garcia et al., 2020)

2.5.3 Diabetes mellitus (DM) and oxidative stress (OS)

Oxidative stress also causes T2DM where ROS impact chemical changes in all cellular components and produce lipid peroxidation, in excess amounts of hydrogen peroxide (H_2O_2), DNA, RNA, and lipids are severely damaged. Catalase (CAT) is the major H_2O_2 regulator, and it neutralizes H_2O_2 by catalytically converting it to water and oxygen (Alam et al., 2022).When catalases levels are deficient, pancreatic islet-cells are more prone to excessive formation of various ROS (reactive oxygen species) that bring about oxidation stress, ultimately leading to pancreatic islet dysfunction and over time T2DM (Alam et al., 2022). The pathogenic mechanisms of diabetic nephropathy involve the generation of ROS, accumulation of advanced glycation end products (AGEs), and the activation of intracellular signaling pathways, including protein kinase C (PKC) . Hyperglycemia-induced activation of the polyol pathway, damage from advanced glycation end products (AGEs), and increased OS have been identified as key contributors to the pathogenesis of peripheral nerve injury .

Oxidative stress is elevated in diabetic state as heme oxygenase, a hallmark protein for oxidative stress detection, is elevated among various tissues of diabetic animals being used in the experiment just a few weeks after the diabetes induction (Kikkawa, 2000). Glucose auto-oxidation , AGE-producing mechanism, mitochondrial dysfunction, and among other are possible sources of ROS which in turn may injure endothelial cell which is linked to the progression of diabetic complications (Kikkawa, 2000)

Polyol pathway activation, non-enzymatic glycation activation, and elevated PKC levels are strongly associated with oxidative stress as a consequence of ROS and in turn fosters micro and macrovascular complications . Stress resulting from ROS plays a role in the inactivation of endothelial nitric oxide synthase, and prostacyclin synthase which are essential anti-atherosclerotic enzymes . Elevated levels of sugar in the blood promote ROS generation, this ROS interacts with deoxyribonucleic acid (DNA) (more so mitochondrial DNA) and various proteins and as a consequence result to cell damage. Mitochondrial superoxide it the main compound responsible for the onset of metabolic abnormalities linked to diabetics. Insulin resistance has been associated with the generation of mitochondrial ROS from free fatty acids and inhibition of anti-atherosclerosis enzymes thereby contributing to atherosclerosis and cardiomyopathy complications progression in diabetes

2.5.4 Advanced glycation products (AGE)

Non-enzymatic glycation of plasma proteins results in the development of AGEs, which alter cellular normal functioning by compromising their molecular structure, enzyme activity disruption, and altering the normal functioning of the receptor. AGEs interact with various cellular components such as, nucleic acids and lipids and as a consequence affects their molecular integrity and function and consequently contribute to the progression of diabetic complications. Plasma proteins that have been corrupted with AGE precursors bind to AGE receptors (RAGE) on macrophages, blood vessels endothelial cells, and blood vessels smooth muscle cells evokes ROS production, this interaction activates the pleiotropic transcription factor, causing multiple pathological alterations in gene expression (Giacco & Brownlee, 2010).

2.5.5 Inflammation

Inflammation is one of the potential risk factors for atherosclerosis and T2DM. Vascular cells undergo a number of pathologic changes in reaction to hyperglycemia, causing monocyte adhesion to vascular ECs. IR plays a role on elevation of tumor necrosis factor alpha and it has inflammatory action. Presence of diabetes have been shown to increase various inflammatory markers like C- reactive proteins, fibrinogen, interleukin, plasminogen activator inhibitor among others, additionally high glucose levels induce activation of monocyte activation which enhances oxidative stress .

2.6 Signs and symptoms of T2DM

Common symptoms linked with diabetes such as polyuria (frequent urination), polydipsia, as well as polyphagia occur commonly in TIDM, which has a rapid development of severe hyperglycemia, and also in T2DM with extremely elevated hyperglycemia (Clark et al., 2007). Losing weight, fatigue, restlessness, and body pain are also common signs of undetected diabetes. The ADA provides a list of seven symptoms: frequent urination, irritability, excessive thirst, unusual weight loss, extreme hunger, increased fatigue, and blurry vision (Clark et al., 2007)

Clark et al. performed a study to Help Improve Early evaluation and management of risk factors Leading to Diabetes (SHIELD) is a 5-year longitudinal observational study of persons with or at risk of getting diabetes which discovered a peculiar opportunity to report the occurrence of the ADA symptoms in various patient groups and the association of those symptoms with diabetes, in which they were all reported more frequently in type 2 diabetic group (Clark et al., 2007)

Of the seven ADA symptoms, those reported most frequently for the previous 12 months by respondents with T1DM and T2DM were frequent urination and increased fatigue, excessive thirst, irritability, and blurry vision ranged across both types of diabetes, erectile/ sexual dysfunction was reported more frequently by those with type 2 diabetes (Clark et al., 2007)

Other non-ADA symptoms, such as erectile dysfunction, shortness of breath, and chest pain, had higher prevalence in the diabetes and individuals who were highly likely to develop diabetes, this can be attributed to their relationship to diabetes or cardiovascular disease and obesity; respondents with the highest BMI (obese) were more likely to report excessive thirst, excessive hunger, irritability, and fatigue but least likely to report sudden weight loss than respondents with lower BMI (normal or overweight) (Clark et al., 2007)

2.7 Diagnosis of T2DM

The current gold standard for diagnosing diabetes is the measurement of glucose in venous plasma, this measurement can be accurate only if glycolysis is inhibited in the blood sample as soon as the sample is drawn, a process that can be achieved in two ways; either the blood tube is stored on ice and the blood is centrifuged within 30 minutes, or glycolysis in the tube is effectively inhibited by appropriate additives (citrate plus fluoride; fluoride by itself is not sufficient) (Kerner & Brückel, 2014)

Diagnostic criteria for diabetes diagnosis is based on values of glucose or glycated hemoglobin (HbA1c) in the plasma, diagnostic cut-off values are presented in Venous plasma glucose is the standard method for measuring and reporting; however, in recognition of the widespread use of capillary sampling, especially in low-resource

settings, values for capillary plasma glucose are provided for post-load glucose values; fasting values for venous and capillary plasma glucose are identical (World Health Organization & International Diabetes Federation, 2006). The following test can be used to diagnose diabetes according to (World Health Organization & International Diabetes Federation, 2006).

Table 0.1: **Tests for Diagnosis of Diabetes Mellitus.**

Test	**Diabetic**
Glycated hemoglobin (HbA1C)	≥6.5%(48mmol/L)
Random plasma glucose test (regardless of the last you ate)	≥200mg/dL(11.1mmol/L)
Fasting venous or capillary plasma glucose	≥126 mg/dL (7 mmol/L)
2- hours post-load venous plasma glucose	≥200 mg/dL (11.1 mmol/L)
2- hours post-load capillary plasma glucose	≥ 220mg/dL (≥ 12.2 mmol/l)

Epidemiological investigations in recent years have shown that the specificity of HbA1 ≥ 6.5 % is high enough to justify a diabetes diagnosis and the sensitivity of HbA1c < 5.7 % is high enough to justify exclusion of a diagnosis of diabetes therefore, HbA1c is suitable as a primary diagnostic tool for excluding diabetes with great certainty and for making a diagnosis of diabetes in some cases; when the HbA1c level lies between 5.7 and 6.4 %, these guidelines recommend that diabetes and prediabetes be diagnosed by measuring glucose in accordance with traditional criteria (Kerner & Brückel, 2014)

In asymptomatic people, the test should be repeated to confirm the diagnosis, preferably with the same test, if plasma glucose ≥18 mmol/L (325 mg/dL), or symptoms are present, urine ketones should be measured to assess the degree of metabolic

disturbance; if plasma glucose measurement is not possible, urine glucose testing can be used to clear diabetes suspicion in people displaying the symptoms in which a negative urine test does not exempt diabetes, but it excludes severe hyperglycemia (World Health Organization & International Diabetes Federation, 2006).

2.8 Complications associated with T2DM

T2DM patients are very susceptible to different forms of both acute and chronic complications. The complications include macrovascular diseases (hypertension, coronary artery disease, heart attacks, hyperlipidemia, strokes, cerebral vascular disease, and peripheral vascular disease), microvascular diseases (retinopathy, nephropathy, and neuropathy) and cancers as illustrated in figure 2.4 (Blahova et al., 2021), Diabetes is at the forefront of causing renal failure, blindness, and amputations besides being the main risk factors associated with stroke and heart disease (Maggio & Pi-Sunyer, 2003).

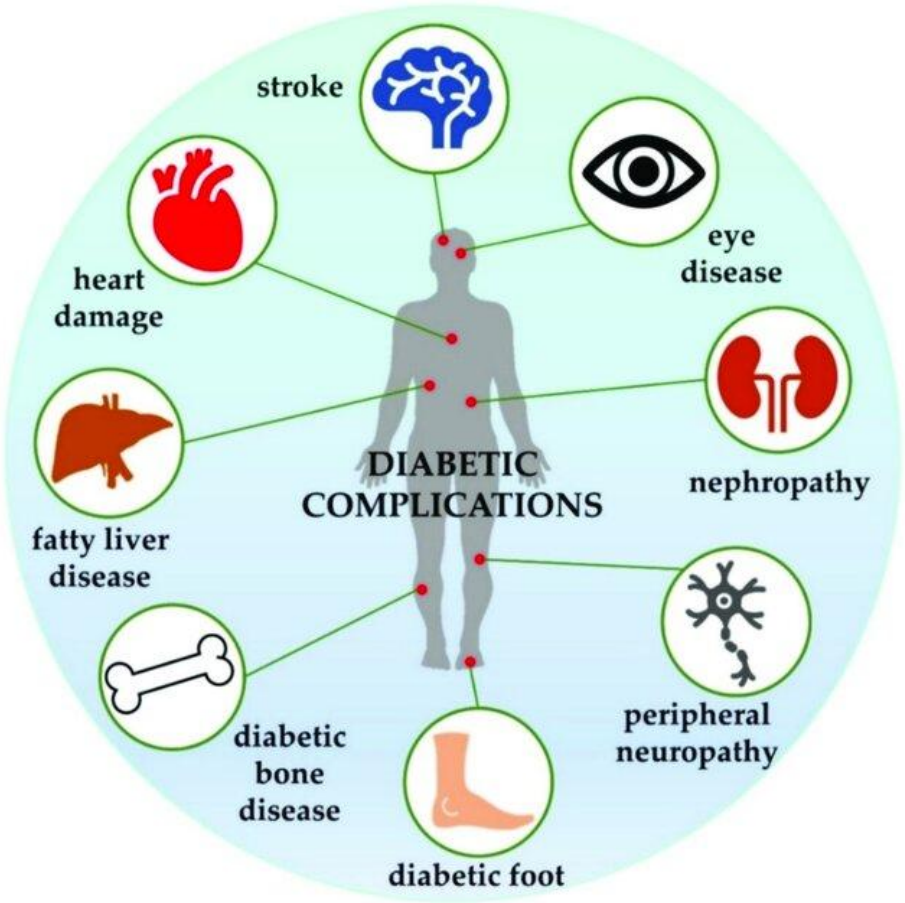

Figure 0.4; Complications associated with T2DM (Blahova et al., 2021)

1.6.1 **Cardiovascular disease (CVD)**

CVD causes up to 65% of all mortalities among diabetic patients (Deshpande et al., 2008) . CVD serves as a leading cause of morbidity and mortality in both prediabetes and T2DM, with OS playing a key role in its pathogenesis by significantly impacting atherogenesis and potentially promoting LDL oxidation (Wu et al., 2014,). Narrowing of blood vessels that carry blood to the arms, legs, stomach, and kidneys results in development of Peripheral Arterial Disease (PAD) in diabetes patients , PAD risk elevates with age advancement, presence of neuropathy as well as duration of diabetes (Deshpande et al., 2008). Premature cardiovascular prevention commonly involves a

synergy of complex treatments with antihypertensive agents, agents that lowers lipid levels and regular low-dose aspirin therapy (Wu et al., 2014,).

2.8.1 Diabetic retinopathy

The primary cause of blindness in adults aged (20–74 years) is diabetic retinopathy and it encompasses various retinal abnormalities such as heightened vascular permeability, excessive neovascularization, capillary microaneurysms, and capillary degeneration (Forbes & Cooper, 2013). Retinopathy causes over 10,000 blindness cases per year and is linked to chronic elevated sugar levels (Deshpande et al., 2008,). Approximately 20% of individuals who develop diabetes exhibit signs of retinopathy development (Blahova et al., 2021; Wu et al., 2014). Prolonged elevated blood sugar levels lead to microvascular damage in the retinal vessels, causing hemorrhage and edema to the retina (Blahova et al., 2021; Wu et al., 2014). Biochemical changes such as oxidative stress, protein kinase C activation, and advanced glycation end product formation have been observed as retinal responses to hyperglycemia (Nentwich & Ulbig, 2015.)

2.8.2 Diabetic nephropathy

Nephropathy (Renal Disease) is a persistent proteinuria (more than 500 mg of protein or 300 mg of albumin per 24 hours) in patients without diseases causing the proteinuria such as urinary tract infection (Deshpande et al., 2008) Nephropathy earliest sign is the presence of small amounts of urinary protein (microalbumin) and cannot be identified through routine urinalysis but can be detected using specialized tests (Wu et al., 2014,). Nephropathy is the leading cause of end-stage renal failure in Western societies (Forbes & Cooper, 2013,)

2.8.3 Diabetic neuropathy

Diabetic neuropathy, a life-threatening complication that involves both peripheral and autonomic nerves, affecting almost half of the diabetic population . Above half of individuals with diabetes ultimately develop neuropathy, with some populations facing a lifetime risk of lower extremity amputation as high as 15% (Forbes & Cooper, 2013,). Diabetic peripheral neuropathy (DPN) is a prevalent complication estimated to affect 30% to 50% of persons having diabetes with primary risk factor being hyperglycemia (Deshpande et al., 2008,). The likelihood of neuropathy development of is directly proportional to both the duration and magnitude of mediated effects on nerve tissue or by endothelial injury or vascular dysfunction; peripheral neuropathy in diabetes appears in several forms depending on the site, manifesting as sensory, focal/multifocal, and autonomic neuropathies; diabetic neuropathy has resulted in more than 80% amputations after foot ulceration or injury and strokes numeric are higher in the neuropathy group .

Diabetic neuropathy can contribute to foot ulcers, amputations, cardiovascular dysfunction, impaired wound healing, and sexual dysfunction (Wu et al., 2014,). Sexual dysfunction, often observed in young diabetic patients, is typically linked to oxidative stress in cavernous tissues (Wu et al., 2014,). There is increasing recognition that caused damage to the spinal cord and higher central nervous system can also occur in diabetes (Forbes & Cooper, 2013,).

2.9 Prevention strategies of T2DM

Many cases of T2DM could be prevented with lifestyle changes, such as regulating body weight, healthy eating, staying physically active, not smoking and drinking

alcohol in moderation (Zheng et al., 2018c). Substituting half an hour of sedentary time with moderate to vigorous physical activity was linked to a 15% increase in HOMA-defined insulin sensitivity (Kolb & Martin, 2017). Physical activity enhances glucose utilization in skeletal muscles by increasing blood flow in the said tissues (Galicia-Garcia et al., 2020). Engagement in heightened physical activeness and increased carbohydrate consumption have been linked to enhanced sensitivity of insulin (Kahn et al., 2006)

Weight loss surgery as a resolution of obesity (for example, bariatric surgery) though costly has proven effective in the prevention and resolution of T2DM (Zheng et al., 2018c). Additionally, intake of vitamin D supplement is considered a promising and cost-effective therapy that may reduce the risk of T2DM and enhance glucose metabolism in diabetic patients (Wu et al., 2014,). Vitamin D effects might be linked to its role in regulation of insulin secretion, insulin sensitivity, and inflammation (Wu et al., 2014,). Besides, even though considered high energy density food, consumption of a handful of nuts daily may provide some protection against T2D (Kolb & Martin, 2017).

Coffee intake (three or more cups daily) lowers by approximately 30 % the risk of T2DM development (Kolb & Martin, 2017). Additionally intake of the same amount of tea, particularly flavonoid rich tea or green tea catechins has been linked to enhancement of blood sugar regulation (Kolb & Martin, 2017). In a study involving 22 entries, it reported that FBS was reduced averagely by 1.4 mg/dL, additionally epidemiological studies further shows that consumption of three or more cups of coffee

or green tea on daily basis lowers the risk of T2DM development by approximately 15% b (Kolb & Martin, 2017).

2.10 Conventional pharmacological interventions of T2DM

Biguanide class of drugs (Metformin, Phenformin and Buformin) function by promoting natural sensation to insulin by decreasing glucose absorption in the intestine, enhancing insulin receptor activity, downregulating gluconeogenesis and upregulating glycolysis . Phenformin and Buformin were associated with elevated incidences of lactic acidosis hence their clinical use was terminated .Metformin, has significantly lower risk hence widely used . Biguanides have been linked to various side effects such as diarrhea, cramps, nausea, vomiting, and increased flatulence, with long term usage being linked to reduced vitamin B12 absorption .

2.10.1 Metformin

The key advantages of metformin include mortality reduction, absence of hypoglycemia risk, an anorexic effect that promotes weight loss; and beneficial effects on lipid concentrations and is particularly suitable for obese, insulin-resistant patients, but it is effective for thin patients as well (Khan et al., 2019; Pfeiffer & Klein, 2014). Metformin is regarded as a safe and reliable interventions in lowering the chances of one getting T2DM, and therefore mostly utilized in prevention and management of diabetes, additionally observation studies have reported that it lowers cancer related mortality in diabetes patients (Khan et al., 2019; Pfeiffer & Klein, 2014).

Metformin induces its effects by acting on the intestines, liver, and kidneys. Metformin reduces FBS levels through mechanisms such gluconeogenesis inhibition, restoring

pancreatic beta cells function and by improving skeletal muscles sensitivity to insulin (Khan et al., 2019). Metformin achieves FBS reduction through inhibition of mitochondrial respiration, which reduces ATP production and activates adenosine monophosphate-activated protein kinase (AMPK, energy sensor enzyme) (Khan et al., 2019). This shift of pathways promotes energy producing pathways and at the same time downregulate energy requiring processes, such as hepatic gluconeogenesis, hence downregulating blood glucose levels (Khan et al., 2019). Metformin also exerts AMPK-independent effects on hepatic gluconeogenesis, such as the inhibition of fructose-1,6-bisphosphatase (Khan et al., 2019).

2.10.2 Incretins

Gastric inhibitory polypeptide (GIP) and Glucagon-like peptide 1 (GLP-1) are among the major compound that stimulate the insulin secretion(Khan et al., 2019). GLP-1 hinders progression of diabetes by enhancing insulin sensitivity and improving the regulation of blood sugar via glucagon secretion suppression and by increasing gut emptying time, this as a consequence reduce the desire for food intake and ultimately resulting in weight loss (Khan et al., 2019). GIP and GLP-1 are rapidly degraded by the enzyme Dipeptidyl Peptidase-IV (DPP-4), and therefore making them ineffective for direct use in the prevention of diabetes mellitus progression (Khan et al., 2019). As a consequence of this limitation, has led to the development of DPP-4 inhibitors and GLP-1 receptor agonists (exenatide and liraglutide) were developed, this agonists cannot be broken down by DPP4 and are therefore able to enhance the effects of incretin hormones since these agonists cannot be broken down by DPP4 and can hence achieve similar results as GLP-1 (Khan et al., 2019).

2.10.3 Sodium-glucose cotransporter (SGLT 2) inhibitors

Some drugs belonging to this group include, empagliflozin, canagliflozin and dapagliflozin, these drugs have been developed recently to treat T2DM. Some of the side effects that have been associated with them include, urogenital tract infection (UTI), genital infection, breast and bladder cancer and hence for more research on them is needed (Khan et al., 2019). They function by inhibiting the operation of the SGLT 2 in the kidneys which are responsible for the glucose reabsorption, therefore aiding in blood glucose regulation (Khan et al., 2019).

2.10.4 Insulin secretagogues

This class of drug therapy exerts its effect by enhancing insulin secretion in the pancreas, they attain this by attaching to the sulfonylurea receptor of ATP-sensitive potassium channel on pancreatic β cells . First-generation sulfonylureas include Acetohexamide, Chlorpropamide, Tolazamide, and Tolbutamide, while second-generation sulfonylureas comprise Glimepiride, Glipizide, and Glibenclamide. Second-generation development of sulfonylureas was driven by their shorter plasma half-life, prolonged duration of effect, faster onset of action, and increased potency . Common side effects associated with this category include symptoms of hypoglycemia, such as sweating, confusion, weight gain, nervousness, dizziness, increased hunger, dark-colored urine, stomach discomfort, and skin reactions

2.10.5 Metiglinide

Includes metiglinide, repaglinide and nateglinide, are derivatives of benzoic acid and belong to the non-sulfonylurea class of insulin secretagogues . They function by closing

ATP-sensitive potassium channels on the plasma membrane of pancreatic β-cells, leading to insulin release .

2.10.6 Amylin analogues

Amylin (deficient in T1DM and T2DM) is a 37-amino acid peptide hormone secreted along with insulin and is responsible in regulating both fasting and postprandial blood glucose levels through being involved in glucose homeostasis by suppressing glucagon secretion and delaying gastric emptying . Amylin has a limitation as a drug since its poorly solubility in solution and tendency to aggregate making it very unstable thus chemical analogs have been established to mimic its function so as to be used in the treatment of both TIDM and T2DM . Pramlintide acetate (only available amylin analogs drug) is administered via the subcutaneous route . Common side effects of amylin analogs include nausea, vomiting, headache, and an increased risk of hypoglycemia when used alongside insulin

2.10.7 Plant-based herbal remedies for T2DM

Several shreds of scientific evidence have proved that certain phytochemicals possess anti-hyperglycemic potentials and can be effectively implicated in the management of diabetic and metabolic complications avoiding notable side effects exerted by conventional drugs (Alam et al., 2022,). Most plants are effective phytochemical herbal medicines and natural antioxidants, in part due to their anti-diabetic compounds, such as phenolic, flavonoids, tannins, and alkaloids that improve the performance of pancreatic tissues by elevating the secretion of insulin or decreasing the intestinal glucose absorption in the alimentary canal (Kooti et al., 2016). Many non-essential nutrients such as phenolics and carotenoids have diverse health beneficial activities

including antioxidant, antiviral and anti-inflammatory properties (Tang & Tsao, 2017). Flavonoids, stilbenes, and alkaloids have been demonstrated to be the major anti-inflammatory natural compounds with potential for further development as new therapies for diabetes and its complications (Kong et al., 2021).

The most common phytochemicals ingredients used in treating diabetes are phenolic, flavonoids, alkaloids, and tannins, these phytochemical exhibit their antidiabetic properties through various mechanisms, such as increasing hormone insulin, increasing glucoses absorption in muscle and adipose tissues, prevention of glucose absorption from the intestine, and prevention of glucose production from liver cells, factors which are mostly responsible for the reduction or elimination of diabetes complications (Kooti et al., 2016). Therapeutic effects achieved by targeting insulin resistance, metabolic inflammation, oxidative stress, and altered gut microbiota, the hallmark of these bioactive phytochemicals and their therapeutic potential lies principally in their anti-inflammatory properties (Kong et al., 2021). Tannin improves the function of pancreatic Beta-cells and increases insulin secretion, quercetin is an antioxidant that acts in several mechanisms related with the removal of oxygen radicals, so prevents lipid peroxidation and metal ion chelation (Kooti et al., 2016). Polyphenols extracted from quinoa have been reported to downregulate IL-1 , IL-8, and TNF cytokines in cultured colonic epithelial Caco-2 cells, and to prevent obesity-induced inflammation and promote gastrointestinal health in mice (Tang & Tsao, 2017)

Resveratrol (a naturally occurring phytoalexin (phenol) present in numerous plant species) has been found being able to stimulate glucose uptake in the absence of insulin. The stimulation of glucose uptake induced by resveratrol seems to be due to increased

action of glucose transporter in the plasma membrane. Studies on rats with experimentally induced diabetes demonstrated increased expression of the insulin-dependent glucose transporter, GLUT4, as a result of resveratrol ingestion, compared with diabetic animals not given resveratrol. Resveratrol is also reported to act as an insulin-secretagogue in different β-cell insulinoma lines which might contribute to its glucose lowering effect (Ahangarpour et al., 2019). Citrus flavonoids have been found to attenuate tissue damage arising from prolonged exposure to elevated glucose levels, mainly by increasing endogenous antioxidants, such as SOD, CAT, and GPx, and reducing the concentration of ROS (Gandhi et al., 2020,). The key molecular mechanisms implicated are, modulation of vital metabolic signaling markers via increasing the expression of IRS-1, PI3K, GSK3β, Akt, and PPARγ, and decreasing the expression of PTP1B, activating and increasing the expression of the imidazoline I-2R, opioid secretion, GLUT4, and IR, and they also modulate the expression of eNOS, MCP-1 and 3, NF-κB, the cytokines TNFα and INFγ, IL1β, IL-2, and IL-6, all of these processes result in the attenuation of inflammatory mediators linked to the pathogenesis and progression of diabetic vascular complications by increasing glucose uptake in peripheral tissues (Gandhi et al., 2020,).

2.10.8 Mondia whitei

Taxonomically, *Mondia whitei* is classified in the family Apocynacea under the subfamily Periplocoideae (Oketch-Rabah, 2012), it is a vigorously growing woody creeper that reaches 6 m in height with leaves that are heart-shaped and spread out on a petiole that is 3–6 cm long, the leaf blades are 10–18 cm long and the flowers are arranged in a panicle with yellow and reddish-purple petals, pictorial representation in

figure 5 & 6 (Oketch-Rabah, 2012). *M. whitei* is widely distributed in tropical Africa from Senegal in the west to southern Sudan in the east and throughout most of Central Africa and South Africa (Oketch-Rabah, 2012). In East Africa, *M. whitei* is popularly known as "mulondo" in Uganda (Oketch-Rabah, 2012) , in Kenya the vernacular Luo name for *M. whitei* is "ogombo," a term that translates to the English word "desire." since among the Luo people, ogombo is believed to awaken life's desires, which include the desire for food, happiness, sex (Oketch-Rabah, 2012). In the western part of Kenya, particularly in Kakamega, *M. whitei* is known as "mukombero" or "mkombelo" and is used to enhance vitality and fertility, as well as to treat rheumatism and stomachache (Oketch-Rabah, 2012).Although *M. whitei* has been used as a medicinal plant for many generations in many countries across Africa, there is a dearth of information regarding the phytochemistry and pharmacology of this plant (Oketch-Rabah, 2012).

2-hydroxy-4-methoxybenzaldehyde a principal tyrosinase inhibitor was isolated and identified from the root of *M. whitei*, besides several alkaloids have also been detected (Oketch-Rabah, 2012), availability of isovanillin (3-hydroxy-4-methoxybenzaldedyde) has been reported (Oketch-Rabah, 2012). An unusual chlorinated coumarin lignan, 5-chloropropacin, along with several of the other previously known benzaldehyde derivatives have also been reported (Oketch-Rabah, 2012). Watcho et al, 2014 studied the phytochemistry of the methanolic and hexane crude extracts of the *M. whitei* root prepared by extraction using a mixture of chloroform and methanol in a 1:1 ratio, in the crude extracts, the authors detected steroids and triterpenes (mixtures of amyrine α- and ß-acetate, lupeol, ß-sitosterol, and ß-sitosterol glucoside). In the hexane crude extract, these researchers found aromatic compounds [2-hydroxy-4 methoxy benzaldehyde, 3-hydroxy-4-methoxy-benzaldehyd (vanillin), and 4-hydroxy-3-methoxy-benzaldehyde],

while methanol crude extract fraction yielded sugars (glucose) and polyholosides [α-D-glucopyranosyl (6-1)-ß-D-glucopyranose and 1-methoxy-ß-D-glucopyranosyl (6-1)-ß-D-glucopyranose] (Oketch-Rabah, 2012). A phenolic glycoside with the sugar 2-O- ß -D-glucopyranolsyl-(1-6)-O- ß –D- xylopyranoside has also been reported (Oketch-Rabah, 2012). *M. whitei* root is rich in sterols and benzyladehyde derivatives and also contains several alkaloids and glycosides (Oketch-Rabah, 2012).In 2010, Neergfaard et al. reported the presence of a monoterpene lactone (−)-loliolide from the leaves of *M. whitei* (Oketch-Rabah, 2012).

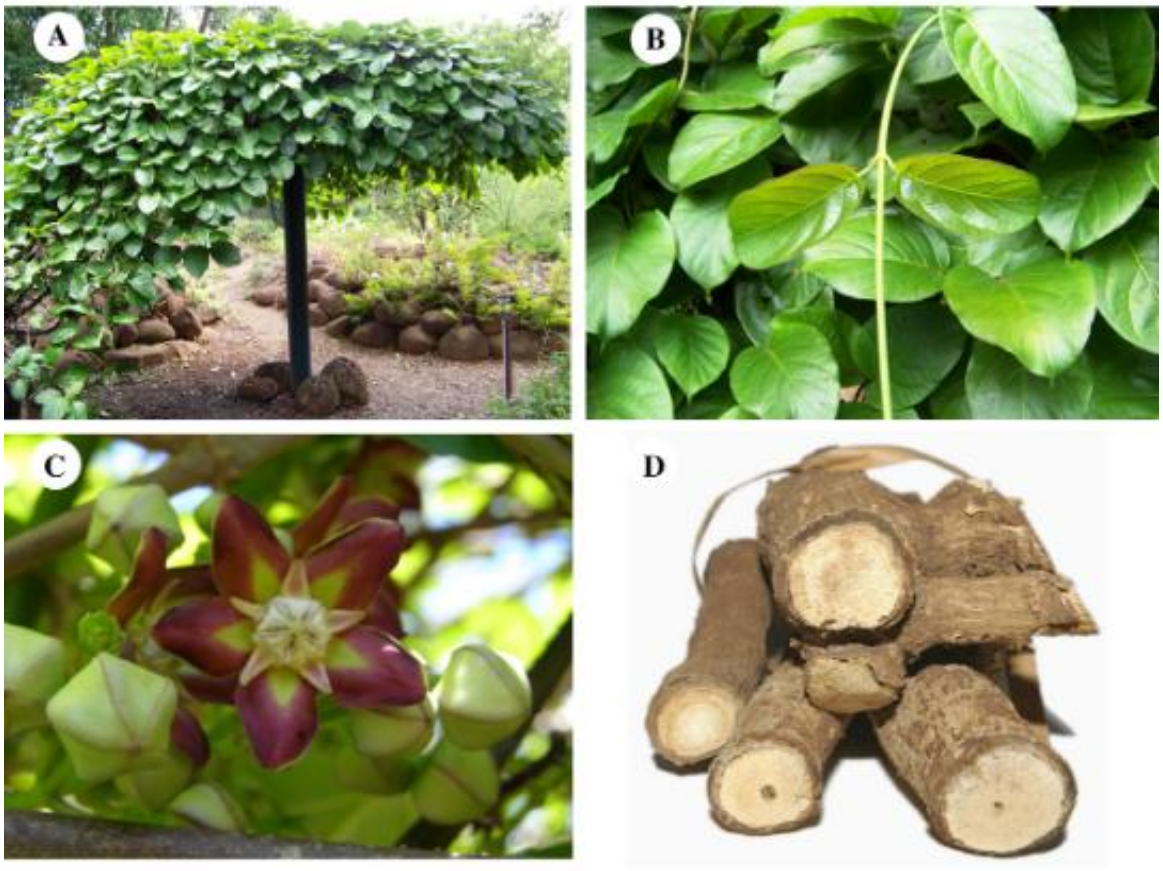

Figure 0.5; *M. whitei* (A) creeping (B) twining stems with leaves;(c) young buds and fully opened flowers and (D) mature roots (Oketch-Rabah, 2012)**.**

Figure:0.6; *M. whitei* roots;

Source https://cuisine228.com/product/herbs/mulondo/.

2.11 Animal models of T2DM

The rodent models have many aspects in common with the human disease, including a number of similarities in genetic loci of susceptibility, influence of the environment and pathogenesis of disease(Bluestone et al., 2010). STZ rats are the more common animal model used to investigate anti-diabetic activity of plant extracts (Kooti et al., 2016) Among several available chemicals used to induce diabetes, STZ is most preferred to model human diabetes in animals, structural, functional and biochemical alterations observed in STZ-induced diabetes resemble those which usually appear with diabetes

in human, therefore STZ-induced diabetes represents a clinically relevant model to study the pathogenesis of diabetes and associated complications in experimental animals (Goyal et al., 2016). Streptozotocin are the most prominent diabetogenic chemicals in diabetes research and is a nitrosourea analogue where the N-Methyl-N-nitrosourea (MNU) moiety is connected to the carbon-2 of a hexose (Lenzen, 2008,) STZ is an antimicrobial besides being used as a chemotherapeutic alkylating agent (Damasceno et al. 2014)

Being a glucose analogue, STZ enters beta-cells via the GLUT2 transporter and accumulates intracellularly once inside the cells STZ forms an alkylating agent, diazomethane (DAM), that causes DNA methylation and elicits diabetogenic action besides (Goyal et al. 2016). In the attempt to repair DNA, poly(ADP-ribose) polymerase (PARP) is overstimulated, this diminishes cellular NAD+, and subsequently ATP, stores, the depletion of the cellular energy stores ultimately results in beta cell necrosis, also protein methylation adds to the functional defects of the beta cells after exposure to streptozotocin (Lenzen, 2008). STZ also induces diabetes via multiple mechanisms such as increased NADPH levels either by glucose auto-oxidation or diacylglycerol (DAG) production and increased O_2^- free radical generation, activation of protein kinase C pathway, glucose flux through the polyol metabolic pathway, accumulation of advanced glycation end products and cytokine secretion, STZ selectively damages b cells allowing STZ to be considered as a unique compound for modeling diabetes in animals with acceptable construct and face validity (Goyal et al., 2016). Streptozotocin (STZ) has been exploited widely in the last three decades to induce diabetes in various animal species and to help screen for hypoglycemic drugs,

STZ induces clinical features in animals that resemble those displayed by diabetes in humans (Goyal et al., 2016).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Materials

Rack Wire cages were used to house the albino rats in this study were assembled in Estates Department, University of Eldoret. Cryovials, vacutainers, needles, syringes, microfine needles, dissecting kit, glucometer (Gluco Rx type), glucose strips (Gluco Rx strips) , automatic pipettes and pipette tips were procured from Bridge Well Scientific, Eldoret, Kenya. Rat pellets (chow) were procured from Maraba Agri Vet in eldoret, Kenya. Streptozotocin (STZ) was procured from Nacalai Tesque, Inc, Kyoto Japan. Thio barbituric acid (TBA) was obtained from (Sigma Aldrich St. Louis, Missouri, USA) and tetramethoxypropane (TMP) was procured from Kobian Scientific, Nairobi, Kenya. DPPH was acquired from Avonchem Wellingtine House Macclesfield, UK. Metformin (Glucophage) was obtained from Lipha Pharma Ltd, UK. Spectrophotometer (Spectro Scan 30, Biotech Engineering Management Co. ltd, UK) was utilized to read absorbance at various wavelengths. All other reagents utilized in this study were of analytical grade.

Ethical considerations

Ethical consent for the animal protocols of this research study was issued by the Research Ethics Committee of University of Eastern Africa, Baraton, Kenya (Reference; UEAB/ISERC/09/06/2023) as highlighted in Appendix III. Research license was acquired from National Commission for Science, Technology & Innovation (License No; NACOSTI/P/24/333020) as highlighted in Appendix II. Also, the experiment was carried out as per the Organization of Economic Cooperation and Development (OECD) Guidance Document on the Recognition, Assessment and

Utilization of Clinical Signs as Human Endpoints for Experimental Animals Used in Safety Evaluation No 19.

3.1 Plant collection and identification

Fresh roots of *M. whitei* were obtained from 0°35’28.0’’N 34°35’42.6’’E, Bungoma County, Kenya and *in situ* identification was done by a local herbalist. *M. whitei* roots were then transported to the University of Eldoret where they were botanically identified by Mr. Dennis Onyango a taxonomist in the Department of Biological Sciences, University of Eldoret, Kenya and was assigned herbarium label number MUH/MOW/03/95.

3.2 Plant crude extract preparation

The roots of *M. whitei* were washed to remove any debris then chopped into small pieces, after which they were dried at room temperature and crushed into homogenous powder utilizing an electric mill (Disk Mill FFc-23, China). Hexane crude extract of *M. whitei* roots was prepared according to (Watcho et al., 2007) whereby the powdered plant (700 g) was soaked in 6 L of CH_2Cl_2:MeOH (1:1) mixture at normal temperature for 72 h after which it was filtered. The filtrate was concentrated at 45 °C using a Rotary Evaporator (Rotavapor type EL 30, model AG CH-9230, Germany) then exhausted for half an hour in 500 mL of hexane, followed by filtration. The solvent was removed utilizing the Rotary Evaporator to remain with paste crude extract which was then weighed. The stock solution (100 mg/mL) was prepared by dissolving a gram of paste in 10 mL distilled H_20 (Watcho et al., 2007). *M. whitei* aqueous crude extract was prepped by dissolving 200 grams of the milled roots in 1.3 L distilled H_20 and stored in the fridge at 4° C for 72 hours, with occasional stirring. Once filtration was done, the solution obtained was put in an oven (50°C) for 72 hours to evaporate the solvent after

which the residue was weighed. Stock solution for aqueous extract was made by dissolving one gram of the residue in 10 mL distilled H_20 for use in subsequent procedures (Watcho et al., 2007). *M. whitei* methanol extract was obtained through maceration of powdered roots using methanol (1:5 w/v) for 72 hours with regular stirring (Kiptisia et al., 2020). The mixture was filtered and concentrated at 50°C using a Rotary Evaporator. (More phytochemicals were detected in methanol and aqueous crude extract compared to hexane crude extract. Methanol extract had higher *in vitro* antioxidants activities compared to aqueous extract, therefore methanol solvent was preferred and consequently employed to obtain the extract used in animals' treatment.)

3.3 Qualitative phytochemical screening of *M. whitei* crude extract

Analysis of phytochemicals in hexane, methanol and aqueous root extracts was done using standard reagents as described previously (Anwar, 2018; Pant et al., 2017; Zohra et al., 2012).

3.3.1 Flavonoids presence test

The crude extract (5 mL) was added to concentrated sulphuric acid (1 mL) and 0.5 g of magnesium. Flavonoids were considered present if a red or pink color that disappeared in three minutes was formed.

3.3.2 Tannins presence test

A milliliter of the crude extract was mixed with 2 mL of water after which three drops of ferric chloride were introduced. Tannis were considered present if a blue green/ black color was formed.

3.3.3 **Saponins presence test**

One milliliter of the crude extract was mixed with 2 mL of distilled H_20 in a test tube, after which the mixture was vigorously shaken. Saponins were considered present if after shaking a froth which persisted for 20 minutes was formed.

3.3.4 **Phenols presence test**

About 2 mg of the crude extract were dissolved in 5 mL of water, after which 3 drops of 5% ferric chloride were introduced. Phenols were considered present if dark green color was formed.

3.3.5 **Alkaloids presence test**

One gram of the extract was dissolved in 2 mL of 0. 1 HCL, after which two drops of Mayer's reagent were introduced. Alkaloids were considered present if a pale-yellow precipitate was formed.

3.3.6 **Coumarins presence test**

One gram of the extract was diluted in 2mL of hot distilled water after which the solution was divided into two portions. 10% NH_4OH (0.5 ml) was added each portion. Double spots developed on filter paper and were analyzed under UV light. With an intense fluorescence indicating presence of coumarins

3.3.7 **Sterols and steroids presence test**

Dissolution of the crude extract was done in 0.5 mL of hot acetic anhydride, where 0.5 mL each of chloroform and Liebermann Burchardt were then added. Blue-green ring formation at the interphase was observed as the positive indicator for the presence of sterols and steroids.

3.3.8 **Terpenoids presence test**

0.5 g of the crude extract was combined with 2 mL of chloroform, shaken and thereafter evaporated. 2 milliliters of concentrated sulphuric acid were added, after which the mixture heated for about two minutes. Development of a greyish color was observed as an indicator of the presence of terpenoids.

3.3.9 **Glycosides presence test**

About 0.5 g of the crude extract was combined with 2 mL of glacial acetic.1 mL of concentrated sulphuric acid was carefully introduced along the side of the test tube. The presence of glycosides' presence was to be indicated by the appearance of a brown ring at the contact.

3.3.10 **Anthraquinones presence test**

About 0.5 g of the extract was agitated with 10 mL of benzene, filtered and integrated with 5 mL of a 10% ammonia solution. After stirring the solution, anthraquinones presence was indicated by the ammonia at the lower phase turning pink, crimson, or violet.

3.4 ***In vitro*** **antioxidant analysis of crude extract**

3.4.1 **DPPH scavenging activity**

DPPH scavenging assay was done according to (Moriasi et al., 2020). 6 mg of a stable free radical, 1,1-diphenyl-2-picryhydrazyl (DPPH), was accurately weighed and placed in 50 mL methanol and made to 0.3 mM solutions. One mL of methanolic solution of 0.3 mM DPPH was added to 2.5 milliliters of each of the methanol and aqueous crude root extract concentrations (10, 50, 100, 200, and 400 µg/mL). The mixtures were shaken followed by incubation for 15 minutes in the dark, at room temperature. Methanol (2.5 mL) plus sample solution (1 mL) was utilized as blank. In addition, L-

ascorbic acid at concentrations equivalent to those of test samples (10, 50, 100, 200, and 400 µg/mL) was utilized as positive control. After incubation, absorbance (A) was determined at 517 nm in triplicate. Percentage of the radical scavenging activity (% RSA) was calculated using the below formula.

$$\%\ \text{DPPH radical scavenging activity} = \frac{\text{Abs of control} - \text{Abs of test sample}}{\text{Abs of control}} \times 100$$

DPPH is a compound that consists of a nitrogen free radical, which is easily quenched by a free radical scavenger. DPPH radicals are reduced into a non-radical form (DPPH-H) when a proton radical scavenger or hydrogen donating antioxidants is present. DPPH radical has an absorbance at 517 nm, which disappears after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule (Patro et al., 2016) .

3.4.2 **Ferric reducing antioxidant power assay**

The ferric reducing antioxidant power of the aqueous and methanolic root extracts of *M. whitei* were evaluated according to the methods described by (Moriasi et al., 2020). The reaction mixtures which incorporated 1 mL of different concentrations of methanolic extract, aqueous extracts and L-ascorbic acid as a positive control (0.2, 0.4, 0.6, 0.8, 1.0, and 5.0 mg/mL), 2.5 milliliters phosphate buffer (200 mM, pH 6.6), and 2.5 milliliters potassium ferricyanide (30 mM) were prepared. The mixtures were then incubated at 50°C for a period of 20 minutes after which 2.5 milliliters of trichloroacetic acid (600 mM) was added, mixed, and followed by centrifugation for 15 minutes at 3000 rpm. Thereafter, 2.5 milliliters of the supernatants were aspirated and stirred with 2.5 milliliters of distilled H_2O and 0.5 mL of $FeCl_3$ (6 mM). Blank was made by taking 1 milliliters of distilled H_2O and treating it as the samples. The absorbance values of

samples and standard were taken in triplicates versus blank at 700 nm utilizing a spectrophotometer. Absorbance values were interpreted as concentration of ferrous ions (Fe^{2+}) formed from reduction of ferric ions (Fe^{3+}) since Fe^{2+} forms a complex-colored solution complex with $FeCl_3$, which is measurable spectrophotometrically at 700 nm. This method measures the ability of analytes to reduce ferric ion at low pH to ferrous ion, yielding a blue colored complex which is measurable at 700 nm where absorbance elevation serves as an indicator of ferric reducing antioxidant power of the analytes (Moriasi et al., 2020).

3.5 Experimental animals

30 male Wistar albino rats (*Rattus norvegicus*) of 6-8 weeks' old were obtained from Zoology Department, Chiromo Campus, University of Nairobi, Kenya. Rats were housed in the racked wire cages with sawdust and soft grass placed at the bottom. Rats were housed in groups of 6 rats under standard laboratory conditions (Temperature, 25°C, 40–60% humidity and 12 h light and 12 h dark cycle) and cleanliness was maintained in the cages. Food and drinking water were accessible to the rats freely. *Rattus norvegicus* (Rats) were allocated two weeks for acclimatization before initiation of the experiments.

3.6 Induction of diabetes mellitus

DM was induced according to (Koech et al., 2020) with slight modification whereby twenty four (24) overnight fasted rats were intraperitoneally issued one injection of 65 mg/Kg bwt of STZ (Szkudelski, 2001) prepared in 100 µL ice cold sodium citrate buffer (0.1 M, pH 4.5) to induce diabetes. Rats in the normal control group were issued an intraperitoneal injection of 100 µL of sodium citrate buffer (0.1 M, pH 4.5). The animals that were injected with STZ were given 5% glucose solution for 12 hours to

overcome drug- induced hypoglycemia. Three days after the injection, hyperglycemia was confirmed by the elevated sugar levels in the blood drawn from tail end using a glucometer. Those whose FBS were ≥150 mg/dl (8.3 mmol/L) (Furman, 2015,) were accepted as diabetic and suitable for study.

Streptomyces chromogens produce the glucosamine–nitrosourea complex STZ, which is utilized in clinical settings as a chemotherapeutic drug to treat pancreatic beta cell cancer (Graham et al., 2011). STZ destroys pancreatic β-cells, by transferring methyl group to the DNA molecule resulting in the fragmentation of the DNA and ultimately hyperglycemia (Graham et al., 2011). The selectivity for β cells is related to the chemical building up in β cells after entering via the GLUT2 glucose transporter receptor, STZ structural resemblance with glucose allows it to bind to GLUT2 receptor (Graham et al., 2011).

3.7 Experimental design

The experimental setup was formulated according to (Chandran et al., 2016) with slight modification. Rats were placed in five groups randomly with each group having six rats as outlined below. The sample size was selected based on the law of diminishing returns and the value "E" was measured, where "E" is the degree of freedom for ANOVA and any sample size with E being greater than or equal to 10 is considered adequate. (Charan & Kantharia, 2013). E was calculated using the below formula:

E = 30 (Total number of animals) – 5 (Total number of groups),

in this case E=25 and therefore the sample was adequate. Rats were randomly categorized in five groups each of six animals as follows;

Group I: Normal rats administered with normal saline daily

Group II: STZ-induced diabetic administered with normal saline daily.

Group III: Low dosage, STZ-induced diabetic rats administered with 250 mg/Kg body weight of crude extract.

Group IV: High dosage, STZ-induced diabetic rats administered with 500 mg/Kg body weight of crude extract.

Group V: STZ-induced diabetic rats treated with 100 mg/Kg body weight of metformin (standard drug).

3.8 Animal treatment

Experimental groups were orally treated at 9 a.m. for 21 days daily with respect to their category of treatment. 100 mg/Kg bwt/day metformin dosage was chosen, while the *M. whitei* methanol crude root extract dosages of 250 and 500 mg/Kg bwt/day per rat were selected (Koech et al., 2020). This regimen of dosages was elected as it had produced noteworthy results in a study conducted by Watcho et al., 2007. Normal saline (0.9 % NaCl) was used as the vehicle and was preferred due to its isotonic property and therefore had reduced osmotic effect. Using an automated micropipette, rats were given oral doses of *M. whitei* crude extract at 250 mg/kg bwt/day, 500 mg/kg bwt/day, and 100 mg/kg bwt/day of metformin every day for 21 days. Animals were fasted for 12 hours prior to taking their FBW and FBS.

3.9 Animal sacrifice and collection of samples

Rats underwent an overnight fasting and thereafter sacrificed in accordance with Ko & Dw (2015) and Parasuraman et al. (2010). All rats were weighed and their FBS ranges were taken on the twenty-second day. Following the administration of chloroform-induced terminal anesthesia, all animals were sacrificed and their hearts punctured using a sterile needle syringe to extract their blood. In order to prevent the heart from collapsing, samples of blood were drawn gradually from the ventricle. To prevent

coagulation, a section of the blood was placed in EDTA-containing vacutainers. The blood underwent centrifugation at one thousand five hundred rpm for 15 minutes to extract plasma for the ensuing *in vivo* antioxidants test. The portions of blood to be utilized in biochemical analysis were drawn in EDTA-free vacutainers, allowed to clot for four hours at 4°C, and followed by centrifugation for fifteen minutes at one thousand five hundred rpm to extract serum. Before the serum was utilized in biochemical tests, it was chilled to -22°C. After blood collection, the animals sacrificed were put on a dissecting board. Vertical midline was cut with a pair of scissors running from the neck to the pelvis to open the peritoneum. The rats' organs, i.e., liver, were excised, washed in ice cold normal saline and kept at -20^0C freezer prior to lipid peroxidation and ferric chloride reducing power assay analyses.

3.10 Biochemical serum analyses of lipid profile and indices of liver and kidney function

The serum obtained after animal sacrifice was used for analysis of the lipid profile that is total cholesterol, triglycerides, High-density lipoprotein-cholesterol (HDL-C), Low-density lipoprotein-cholesterol (LDL-C). Liver function tests were done by analyzing serum levels of GGT, ALP, AST, ALT, total bilirubin, bilirubin direct, total proteins, and albumin (ALB). Kidney function tests were done by analyzing the levels of urea and creatinine in the serum. These analytical tests were carried out in accordance to standard functioning protocols of Cobas Integra 400 plus auto-analyzer (Roche Diagnostics, Mannheim, Germany) at MTRH, Eldoret. Cobas Integra 400 plus auto-analyzer principle of functioning is anchored to the principles of turbidimetric, photometric, and ion-selective electrode (ISE). Each assay relies on certain reactions of chemicals that produce quantifiable change in color, which is correlated to

concentration of the analyte in the sample. Lipid profile assays for example, these assays depend on the conversion of the analyte into a detectable product by utilization to the enzyme, this product can be quantified by measuring its absorbance at specific wavelength photometrically. As for liver function test assay, catalytic action of various enzymes such as ALT, AST and ALP on specific substrates are determined as they lead to the development of chromogenic products, which can be quantified by absorbance. As for the case kidney function tests, serum urea interacts with selected reagents resulting in a quantifiable color change, which is detectable photometrically.

3.11 *In vivo* antioxidant capacity analyses

3.11.1 Effect of *M. whitei* extracts on plasma's potency to reduce ferric ions

Reducing potency of plasma was determined in accordance with the ability of their antioxidant capacity to form colored complex with potassium ferricyanide, TCA and $FeCl_3$. Effect of extracts on plasma reducing power was determined according to (Merghem et al., 2019). 1 mL of plasma was mixed with 0.5 mL phosphate buffer (0.2 M, pH 6.6) and 0.5 mL potassium ferricyanide (1% w/v). The mixture was incubated at 50°C for 20 min. Termination of the reaction was done by adding 2 mL trichloroacetic acid (10% w/v), followed by centrifugation at 3000 rpm for ten minutes. After centrifugation, 0.5 mL supernatant was mixed with 0.5 mL distilled water and 0.1 mL $FeCl_3$ (0.1% w/v). After 5 min, absorbance was determined at 700 nm. A mixture containing all the above reagents except the sample was utilized as blank. Absorbance values were interpreted as concentration of ferrous ions (Fe^{2+}) formed from reduction of ferric ions (Fe^{3+}) since Fe^{2+} forms a complex (colored) with $FeCl_3$, which can be measured spectrophotometrically at 700 nm.

3.11.2 **Effect of *M. whitei* extract on liver's ability to reduce ferric chloride**

This assay was done according to (Merghem et al., 2019) with little modifications. Ten percent (w/v) liver homogenate was developed in Tris HCL buffer (pH 7.4), followed by centrifugation at 4000 rpm at 4 °C for 15 min after which supernatant collected. 1mL of supernatant was combined with 0.5 mL phosphate buffer (0.2 M, pH 6.6) and 0.5 mL potassium ferricyanide (1% w/v). The mixture was incubated at 50°C for 20 min. After terminating the reaction by adding 2 mL trichloroacetic acid (10% w/v), the solution then put under centrifugation at 3000 rpm for ten minutes. 0.5 mL supernatant of the resultant solution was combined with 0.5 mL distilled H_20 and 0.1 mL $FeCl_3$ (0.1% w/v). After 5 min, absorbance was taken at 700 nm. A mixture containing all the above reagents except the sample was utilized as blank. Absorbance values were interpreted as concentration of ferrous ions (Fe^{2+}) formed from reduction of ferric ions (Fe^{3+}) since Fe^{2+} forms a colored complex of ferric chloride, which is measurable spectrophotometrically at 700 nm

3.12 Lipid peroxidation of the liver tissues

Malondialdehyde (MDA), a hallmark of peroxidation of the lipids of liver tissues was analyzed as outlined by (Koech et al., 2020) with a little modifications. The liver tissues, initially frozen at -20°C, were allowed to acclimatize to indoor temperature for an hour. After homogenizing one gramme of the extracted liver tissue in nine milliliters of 1.15% cold KCl using a mortar and pestle, the resulting 0.1 mL of supernatant was added to 0.2 milliliters of 8.1 percent sodium dodecyl sulphate (SDS), 1.5 milliliters of 20 percent acetic acid, and 1.5 milliliters of 8 percent Thio barbituric acid (TBA). The mixture's volume was increased to 4 milliliters with distilled water, then heated to 95°C for an hour utilizing boiling chips. Tubes were then allowed to cool to indoors

temperature before their final volumes were adjusted to 5 mL each. After adding five milliliters of the butanol: pyridine (15:1) mixture, the solution was thoroughly agitated for two minutes. The mixture was centrifuged at 400 rpm for ten minutes, after which the top layer was taken, and its optical density was measured at 532 nm using a spectrophotometer. Hydrolyzed 1,1,3,3-tetramethoxypropane (TMP) was utilized as a standard to generate a calibration curve which was used to determine MDA levels in the sample analytes. TMP was treated as the sample by the addition of the same reagents added in the sample, standard concentrations of 5- 100 µM were prepared and made to 10 mL. TMP was used to developed MDA calibration curve as follows, 1.667 mL of 6M TMP was made by diluting 100 mL of distilled water to create 100 mM stock. By diluting 50 µL of the 100 mM solution to 50 mL with distilled water, this resulting mixture was then utilized to make 100 µM stock solutions. As shown in Appendix I, the resulting solution was utilized to create a standard calibration curve for MDA analysis by creating series of 10 µM, 20 µM, 40 µM, 60 µM, 80 µM, and 100 µM solutions. 0.1 mL of 1.15% KCl served as the reference blank, which was prepared by adding the same reagents as the samples and standards. Finally, MDA amount in the samples was expressed in µM. This technique relies on the production of MDA, a pink chromogen that can be detected at 532 nm, as a byproduct of lipid peroxidation that interacts with Thio barbituric acid to produce Thio barbituric acid reactive substance (TBARS).

3.13 Data management and statistical analysis

Data collected was recorded, stored and managed in MS Excel and then fed into the R software, where further data analysis and post hoc analysis was done. Statistical analysis was attained by utilizing MS Excel and R software. Means and standard error

of means (SEM) were calculated through MS Excel. Statistical differences were done by paired Student's t test and One Way Analysis of Variance (ANOVA) at the confidence limit of 95%.

CHAPTER FOUR

RESULTS

4.1 Phytochemical composition of *M. whitei* crude root extract

Aqueous, hexane, and methanol solvents were used to obtain crude extracts from *M. whitei* roots, phytochemical analysis was done, and the outcomes are indicated in Table 4.1. In aqueous extract, saponins, phenols, tannins, flavonoids, glycosides, coumarins, steroids/sterols, and terpenoids were detected however alkaloids and anthraquinones were not detected. In hexane extract, saponins, phenols, tannins, glycosides, coumarins, steroids/sterols, and terpenoids were detected however alkaloids, flavonoids and anthraquinones were not detected. In the methanol extract, saponins, phenols, tannins, alkaloids, flavonoids, glycosides, coumarins, steroids/sterols, and terpenoids were detected however anthraquinones were not detected. Given the superior phytochemical yield in the aqueous and methanol extracts, these solvents were deemed more suitable for subsequent *in vitro* antioxidant testing.

Table 0.1: The qualitative phytochemical screening of *M. whitei* crude root extract.

Phytochemicals	Aqueous extract	Hexane extract	Methanol extract
Saponins	+	+	+
Phenols	+	+	+
Tannins	+	+	+
Alkaloids	-	-	+
Flavonoids	+	-	+
Glycosides	+	+	+
Coumarins	+	+	+
Steroids/Sterols	+	+	+
Terpenoids	+	+	+
Anthraquinones	-	-	-

Presence (+), Absence (-).

4.2 *In vitro* anti-oxidant properties of the *M. whitei* crude root extract

4.2.1 DPPH scavenging activity

Methanolic and aqueous extracts exhibited a remarkable concentration-dependent increase in percentage of DPPH scavenged (Figure 4.1). Absorbance values were taken at wavelength 517 nm. Across all tested concentrations methanolic extract had higher scavenging activity than aqueous extract. L - ascorbic acid (positive control) had higher absorbance than methanolic and aqueous extract. ANOVA analysis pointed to a significant difference ($p < 0.05$) between the extracts and L-ascorbic acid at various concentration. Inhibitory concentration (IC_{50}) values, representing the concentration required to scavenge 50% of the DPPH radicals, were 23.00 µg/mL, 42.71 µg/mL and 116.79 µg/mL for ascorbic acid, methanol extract and aqueous extract respectively.

The aqueous extract had the highest IC_{50} value, while the methanol extract exhibited a lower IC50 value than the aqueous extract, on the other hand ascorbic acid showed the lowest IC_{50} value compared to both the methanol and aqueous extracts

Table 0.2: DPPH scavenging activity IC_{50} Values.

Analyte	Ascorbic acid	Methanol Extract	Aqueous extract
IC_{50} µg/mL	23.00	42.71	116.79

IC_{50}- Half-maximal inhibitory concentration**.** Samples with IC50 values under 50 µg/mL are categorized as very strong antioxidants, those between 50 and 100 µg/mL as strong antioxidants, values from 101 to 150 µg/mL as moderate antioxidants, and values above 150 µg/mL as weak antioxidants (Moriasi et al., 2020).

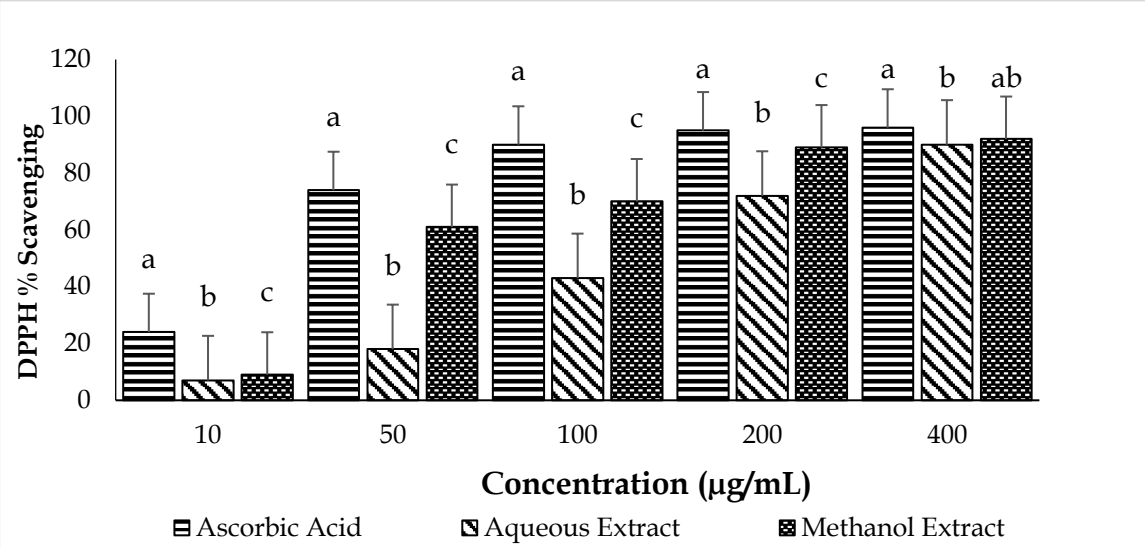

Figure 0.1: DPPH free radial scavenging activity of *Mondia whitei* root extracts. Values are presented as mean ± SEM; n=3. Bars with varying alphabets have differences that are significant ($p < 0.05$, ANOVA). DPPH–1-diphenyl-2-picrylhydrazyl.

4.2.2 Ferric ions reducing antioxidant power (FRAP) assay

Methanolic and aqueous extracts exhibited concentration-dependent increases in absorbance values at wavelength 700 nm which was an indication of the extracts' reducing power (Figure 4.2.). L- ascorbic acid (positive control) exhibited remarkably higher absorbance than methanolic and aqueous extract. ANOVA analysis revealed a significant difference between the extracts and L-ascorbic acid at various concentration. At 5 mg/mL concentration, disparities were significant between methanolic and aqueous extract ($p < 0.05$, ANOVA). Inhibitory concentration (IC_{50}) values, representing the concentration required to achieve 50% reduction in ferric ions, were 0.23 mg/mL, 24.13 mg/mL and 29.5 mg/mL for ascorbic acid, methanol extract and aqueous extract respectively as summarized in table 4.3. Ascorbic acid had the lowest IC_{50}, while that of methanol was lower than aqueous. The methanolic extract showed a considerably higher reducing power compared to the aqueous extract, reflecting its greater antioxidant capacity, based on this methanol was selected to obtain extract used in subsequent animal studies.

Table 0.3: FRAP assay IC_{50} values

Analyte	Ascorbic acid	Methanol Extract	Aqueous extract
IC_{50} mg/mL	0.23	24.13	29.5

IC_{50}- Half-maximal inhibitory concentration. Samples with IC_{50} values under 50 µg/mL are categorized as very strong antioxidants, those between 50 and 100 µg/mL as strong antioxidants, values from 101 to 150 µg/mL as moderate antioxidants, and values above 150 µg/mL as weak antioxidants (Moriasi et al., 2020).

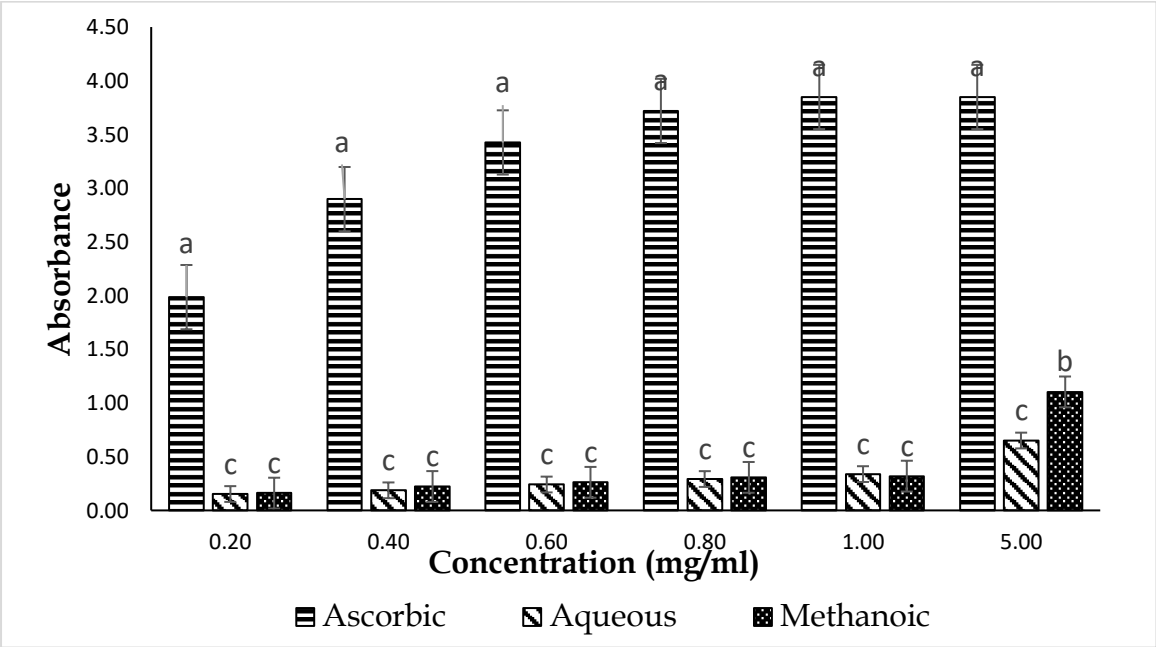

Figure 0.2: Ferric ions reducing power of *Mondia whitei* root extracts. Values are presented as the mean ± SEM; n=3. Bars with varying alphabets have differences that are significant ($p < 0.05$, ANOVA).

4.3 Effects of *M. whitei* crude root extract on FBS

Fating blood sugar (FBS) levels were determined at baseline (before induction), 0 Day (after induction) and weekly thereafter for 21 days in diabetic rats (STZ-induced) as well as non-diabetic controls. FBS levels at baseline were used to confirm normal sugar concentration in the blood of all the rats before induction, while hyperglycemia was confirmed three days after induction with STZ at Day 0. As highlighted in Figure 4.3, normal control category exhibited stable FBS levels which were within the normal range (to 3.73 ± 0.09 to 4.50 ± 0.23 mmol/L) throughout the study period. In the diabetic control group FBS significantly went up from 19.57 ± 1.87 mmol/L at day zero to 26.90 ± 1.11 mmol/L at the 21st day. Once treatment was introduced FBS ranges in the groups that were subjected to treatment significantly decreased while in the diabetic untreated group FBS ranges continued to elevate throughout the study. Treating the rats with the

200 and 400 mg/Kg bwt of *M. whitei* crude root extract reduced FBS ranges significantly compared to the diabetic untreated group. In the group under 200 mg/Kg bwt treatment FBS range declined from 19.33 ± 0.43 mmol/L at day zero to 12.03 ± 0.18 mmol/L at the 21st day. A similar trend was observed in the group under 400 mg/Kg bwt of *M. whitei* crude root extract where the FBS range dropped from 19.35 ± 1.59 mmol/L to 11.60 ± 0.62 mmol/L within the same span of treatment. In the group that was under 100 mg/Kg bwt metformin treatment, a more pronounced drop in FBS range was experienced as evidenced by FBS levels drop from 19.80 ± 0.46 mmol/L at day zero to 10.23 ± 0.78 mmol/L by the 21st day. Analysis done using ANOVA ($p < 0.05$) indicated significant differences between the treatment group and both normal and diabetic control. Further probe into the data collected by doing a comparison using Tukey's HSD test insighted that the FBS ranges in all the categories under treatment decreased significantly by the 21st day compared to the diabetic group with the higher dose of *M. whitei* crude root approaching the effectiveness of the metformin group.

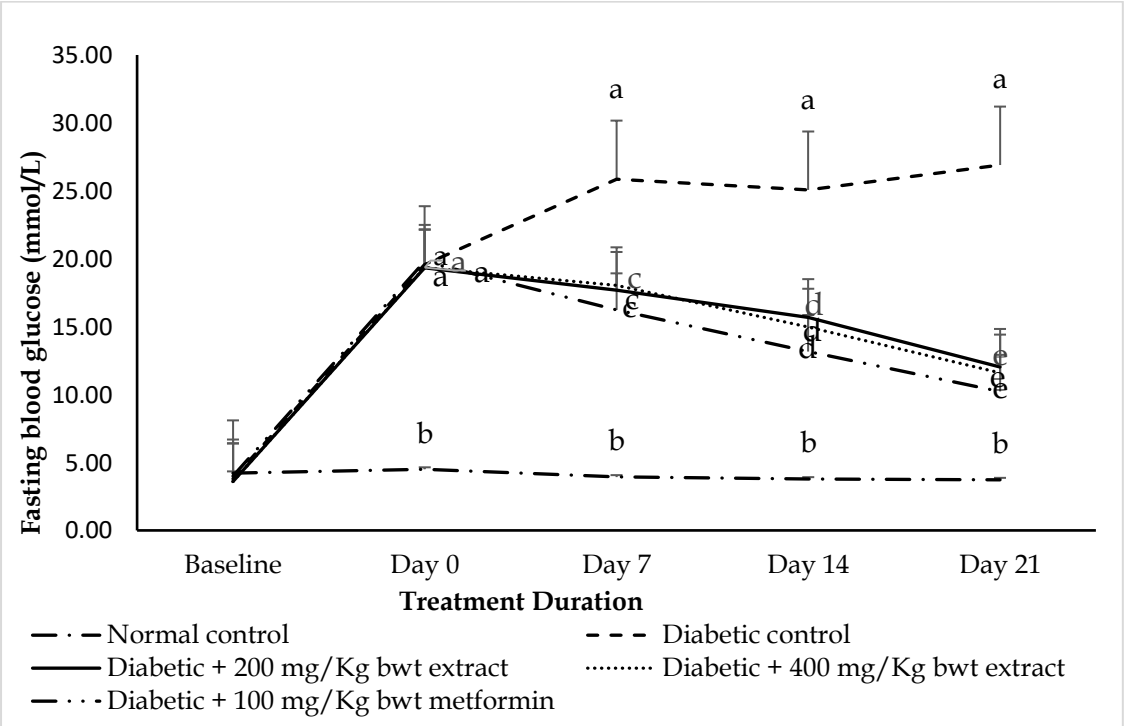

Figure 0.3*:* Effects of *Mondia whitei* crude root extract on fasting blood sugar levels in STZ-induced diabetic rats. Values are presented as mean ± SEM, n = 6. Values with superscript c, d & e are significantly different compared to diabetic control while the values with superscript a, c, d & e are significantly different compared to normal control ($p < 0.05$, ANOVA). Baseline values represent the mean values before induction of diabetes.

4.4 Effects of *M. whitei* crude root extract on body weights

Effects of *M. whitei* crude root extract on body weights were keenly monitored over a 21-day period in STZ-induced diabetic rats' category and normal control as highlighted in Table 4.4. The normal control category showed a slight increase in body weight from 153.50 ± 4.63 g to 164.00 ± 5.49 g (7% increase) over the same period, indicating normal growth while the body weights of diabetic animals dropped. In diabetic control category, body weight significantly decreased from 147.67 ± 2.67 g at day 0 to 125.33

± 3.76 g (15% decrease) at day 21 ($p < 0.05$, ANOVA). Treatment of diabetic rats with *M. whitei* crude root extract at 200 mg/Kg bwt and 400 mg/Kg bwt resulted in a moderate reduction in body weight relative to the untreated rats in diabetic control group. The 200 mg/Kg bwt group experienced a decrease in body weight from 155.67 ± 7.86 g at day 0 to 133.67 ± 4.81 g (14% decrease) at Day 21, while the 400 mg/Kg bwt group showed a reduction from 144.00 ± 4.03 g to 124.25 ± 6.17 g (13% decrease) within the same period. Despite these decreases being significant statistically compared to the normal control, severity was less than the weight loss observed in the rats in diabetic control category. The group treated with metformin (100 mg/Kg bwt) also experienced a significant decrease in body weight from 145.00 ± 2.89 g to 126.33 ± 1.76 g (13% decrease), but this was not significantly different compared to the weight loss experienced in the 400 mg/Kg bwt *M. whitei* group. ANOVA analysis revealed significance in differences of fasting body weights between the treatment groups and both the diabetic and normal controls ($p < 0.05$).

Table 0.4 Effects of *M. whitei* crude root extract on body weights (g).

Treatment group	Day 0_FBW (g)	Day 7_FBW (g)	Day 14_FBW (g)	Day 21_FBW (g)	% Change in body weight on 21st day (from day 0)
Normal control	153.50 ± 4.63	153.50 ± 2.99	154.00 ± 2.94	164.00 ± 5.49	+ 7
Diabetic control	147.67 ± 2.67	141.33 ± 2.03	135.00 ± 4.04	125.33 ± 3.76[a]	- 15
Diabetic + 200 mg/Kg bwt extract	155.67 ± 7.86	159.00 ± 7.23	145.67 ± 6.89	133.67 ± 4.81[a]	- 14
Diabetic + 400 mg/Kg bwt extract	144.00 ± 4.03	135.00 ± 7.09	135.50 ± 7.09	124.25 ± 6.17[a]	- 13
Diabetic + 100 mg/Kg bwt metformin	145.00 ± 2.89[a]	138.00 ± 1.73[a]	134.33 ± 4.10[a]	126.33 ± 1.76[a]	- 13

Values are presented as mean ± SEM, n=6. Values with superscript are considerably different in comparison to diabetic and normal control. ($p < 0.05$, ANOVA). FBW, Fasting Body Weight.

4.5 Effects of crude *M. whitei* root extract on liver and kidney function serum indices

The impact of *M. whitei* crude root extract on liver and kidney function was assessed by measuring various serum indices. For kidney function, tests included urea and creatinine, while for liver function, tests included total bilirubin, direct bilirubin, gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total protein, and albumin. Results are summarized in Table 4.5.

Normal control category exhibited significantly lower levels of bilirubin and liver enzymes such as ALT, AST, GGT, and ALP, as compared to the diabetic categories. Diabetic control category exhibited elevated amounts of total bilirubin, direct bilirubin, and liver enzymes (ALT, AST, GGT, and ALP). Treatment with *M. whitei* significantly reduced these levels. For instance, treatment with *M. whitei* at 200 mg/Kg bwt reduced ALT amounts by 44.5% (106.80 ± 6.30 U/L) demonstrating greater efficacy in restoring normal liver function while the 400 mg/Kg bwt dose achieved a reduction of 22.7% (148.93 ± 5.13 U/L) as metformin illustrated a 31.5% reduction (131.88 ± 4.96 U/L) compared to ALT amounts in diabetic control. Diabetic control category showed AST levels of 253.20 ± 14.75 U/L. Treatment with *M. whitei* at 200 mg/Kg bwt reduced AST levels by 3.0% (245.70 ± 4.65 U/L) and in the category treated with 400 mg/Kg bwt group, AST levels increased by 33.7% (338.60 ± 14.36 U/L). Metformin, however, showed no change (252.60 ± 8.34 U/L). For GGT, the diabetic control category exhibited elevated levels of 11.33 ± 0 .88 U/L and treatment with *M. whitei* at 200 mg/Kg bwt led to a 64.7% reduction (4.00 ± 0.58 U/L) while the 400 mg/Kg bwt dose reduced GGT levels by 38.2% (7.00 ± 0.41 U/L) and metformin reduced GGT by 49.3% (5.75 ± 0.48 U/L).

In the normal control group total protein and albumin serum levels were higher than in the diabetic groups. In the groups under various treatment regimen, total protein and albumin ranges in the serum elevated compared to the diabetic control. In the group under 200 mg/Kg bwt *M. whitei* treatment total proteins levels were elevated to 66.37 ± 3.35 g/L which is a 11.4 % increase within the 21 days of duration, while in the group under 400 mg/Kg bwt *M. whitei* total proteins ranges went up to 59.60 ± 2.51 g/L which is a slight 0.1 % increase. Metformin administration led to an increase of up

to 63.13 ± 3.67 g/L which is a 6 % elevation of the total protein's levels in the serum. The level of albumin in the diabetic control category was 28.50 ± 1.25 g/L. Treatment with 200 mg/Kg bwt *M. whitei* regimen elevated albumin levels to 34.13 ± 1.63 g/L which is a 19.8 % increase while the 400 mg/Kg bwt *M. whitei* regimen elevated albumin levels to 31. 90 ± 0.66g/L which is a 11.9 % increase, metformin administration also increases albumin levels to 33.95 ± 3.42 g/L which is a 19.1 % increase.

Creatinine and urea levels which were investigated as indicators of kidney function, urea ranges were lower in the normal control compared to the diabetic categories, however creatinine levels were higher in normal control relative to the diabetic groups. In the rats belonging to diabetic control category, urea levels were elevated compared to the groups under treatment. Treatment with *M. whitei* at 200 mg/Kg bwt reduced urea levels by 17.5% (19.47 ± 0.56 mmol/L), and with 400 mg/Kg bwt treatment, the reduction was 4.2% (22.60 ± 1.16 mmol/L), while metformin at 100 mg/Kg bwt showed a more substantial reduction in urea level by 19.9% (18.90 ± 1.35 mmol/L). Creatinine levels in animals belonging to diabetic control category were 22.00 ± 2.52 mmol/L. Treatment with *M. whitei* at 400 mg/Kg bwt reduced creatinine levels by 8.0% (20.25 ± 0.63 mmol/L) and in the category treated with *M. whitei* at 200 mg/Kg bwt creatinine levels increased by 3.0% (22.67 ± 1.45 mmol/L). In the category treated with metformin at 100 mg/Kg bwt creatinine levels increased by 6.8% (23.50 ± 1.04 mmol/L). Student's t-test indicated that the reductions in liver enzymes in *M. whitei* treated groups were statistically considerable when contrasted to diabetic control category ($p < 0.05$).

Table 0.5: Effects of crude *M. whitei* root extract on liver and kidney function serum indices

Biochemical parameters	Normal control	Diabetic Control	Diabetic + 200 mg/Kg bwt extract	Diabetic + 400 mg/Kg bwt extract	Diabetic + 100 mg/Kg bwt metformin
Total bilirubin (mmol/L)	1.20 ± 0.15	1.50 ± 0.15	1.40 ± 0.06	2.13 ± 0.06[a]	1.65 ± 0.06[b]
Bilirubin direct (mmol/L)	0.58 ± 0.10	1.17 ± 0.12	0.63 ± 0.03[c]	1.38 ± 0.05[b]	0.68 ± 0.05
GGT (u/L)	2.75 ± 1.11	11.33 ±0 .88[a]	4.00 ± 0.58[d]	7.00 ± 0.41[b]	5.75 ± 0.48[d]
AST (u/L)	181.43 ± 8.24	253.20 ± 14.75[b]	245.70 ± 4.65[b]	338.60 ± 14.36[b]	252.60 ± 8.34[b]
ALT (u/L)	66.13 ± 3.12	192.60 ± 15.13[a]	106.80 ± 6.30[c]	148.93 ± 5.13[a]	131.88 ± 4.96[a]
ALP (u/L)	94.50 ± 5.30	388.33 ± 12.39[a]	192.67 ± 13.86[c]	260.25 ± 7.70[c]	280.00 ± 8.71[c]
Total Protein (g/L)	73.58 ± 3.28	59.57 ± 5.77	66.37 ± 3.35	59.60 ± 2.51	63.13 ± 3.67
Albumin (g/L)	41.93 ± 1.67	28.50 ± 1.25[b]	34.13 ± 1.63	31.90 ± 0.66[b]	33.95 ± 3.42
Urea (mmol/L)	8.13 ± 0.46	23.60 ± 2.51[b]	19.47 ± 0.56[b]	22.60 ± 1.16[b]	18.90 ± 1.35[b]
Creatinine (mmol/L)	30.50 ± 1.76	22.00 ± 2.52[b]	22.67 ± 1.45[b]	20.25 ± 0.63[b]	23.50 ± 1.04[b]

Values represent mean ± SEM; n =6. Mean values with letters a, c, d, e are significantly different compared to diabetic control while those with letter a, b & e are significantly different compared to normal control. ($p < 0.05$, Student's t test). GGT- gamma-glutamyl transferase, AST-aspartate transaminase, ALT-alanine transaminase, ALP-alkaline phosphatase.

4.6 Effects of *M. whitei* crude root extract on serum lipid profile parameters

The impact of *M. whitei* crude root extract on serum lipid profiles was evaluated by measuring key parameters, including triglycerides, total cholesterol, HDL-C, and LDL-C, across different treatment groups. Analysis results are summarized in Figure 4.4.

Normal control had lower triglycerides (TG) levels compared to diabetic animals with exception to category of rats that underwent 400 mg/Kg bwt *M. whitei dose* treatment. The diabetic control group showed elevated triglyceride levels (1.40 ± 0.15 mmol/L) compared to the normal control group (0.95 ± 0.12 mmol/L). In comparison to the diabetic control group, treatment with *M. whitei* at 200 mg/Kg bwt resulted in an unexpected increase in triglyceride levels to 2.01 ± 0.20 mmol/L (43.6% increase). While at the same time, the 400 mg/Kg bwt dose of *M. whitei* significantly reduced triglycerides by 59.3% to 0.57 ± 0.10 mmol/L ($p < 0.05$), suggesting a dose-dependent effect of the extract on lipid metabolism. Metformin treatment reduced triglyceride levels by 23.6% to 1.07 ± 0.10 mmol/L, indicating that the higher dose of *M. whitei* was slightly more effective in correlation to metformin in reducing triglycerides.

Normal control had higher levels of total cholesterol compared to the groups under metformin and 400 mg/Kg bwt of *M. whitei* treatment, but lower than diabetic control and group on 200 mg/Kg bwt of *M. whitei* treatment. Total cholesterol levels declined in the groups under metformin and 400 mg/Kg bwt of *M. whitei* treatment but increased in the group under 200 mg/Kg bwt of *M. whitei* treatment compared to diabetic control group (1.36 ± 0.08 mmol/L). Treatment with *M. whitei* at 400 mg/Kg bwt reduced total cholesterol by 14% to 1.17 ± 0.06 mmol/L, while metformin treatment reduced

cholesterol by 12.5% to 1.19 ± 0.05 mmol/L. These reductions suggested that at high dosage *M. whitei* treatments had potency to regulate total cholesterol levels.

HDL-C levels are known to be protective against cardiovascular disease and for normal control, it had higher HDL-C levels (0.76 ± 0.05 mmol/L) compared to diabetic animals. HDL-C levels in diabetic control were lower than in the treatment groups. Treatment with *M. whitei* at 200 mg/Kg bwt significantly increased HDL-C levels by 52.6% to 0.87 ± 0.07 mmol/L, while the 400 mg/Kg bwt dose-maintained HDL-C levels close to the normal control (0.59 ± 0.06 mmol/L). Metformin increased HDL-C by 5.3% to 0.60 ± 0.05 mmol/L, showing that *M. whitei* at 200 mg/Kg bwt had a more pronounced effect on HDL-C than metformin. For LDL-C, often referred to as "bad cholesterol," the normal category had lower levels compared to diabetic groups. LDL-C level was highest in the diabetic control group (0.39 ± 0.04 mmol/L). Treatment with *M. whitei* at 400 mg/Kg bwt reduced LDL-C by 10.0% to 0.36 ± 0.03 mmol/L, approaching the levels seen in normal control category (0.26 ± 0.03 mmol/L). However, metformin was more effective, reducing LDL-C by 30.0% to 0.28 ± 0.02 mmol/L, indicating that metformin remains more effective than *M. whitei* in reducing LDL-C levels.

Student's t test showed that the reductions in triglycerides, total cholesterol, and LDL-C, additionally increases in HDL-C in the *M. whitei* treatment groups, were significant statistically when compared to the diabetic control category ($p < 0.05$).

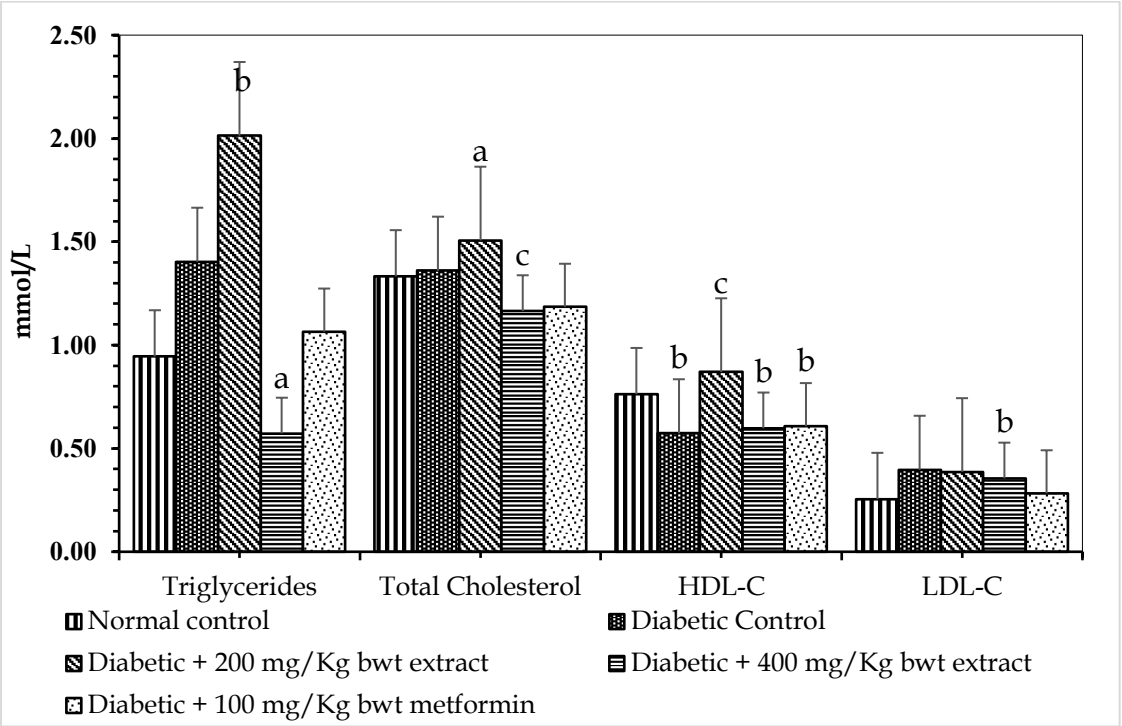

Figure 0.4: Effects of *Mondia whitei* crude root extract on serum lipid profile parameters in STZ-induced diabetic rats. Values represent mean ± SEM; n =6. Mean values with letter a, c on bar graphs is significant statistically compared to diabetic control & while those with letter a & b, are significantly different in comparison with the normal control ($p < 0.05$, Student's t-test). HDL-C; High-density lipoprotein-cholesterol, LDL-C; Low-density lipoprotein-cholesterol.

4.7 Effects of *M. whitei* crude root extract on liver tissue and blood plasma capacity to reduce ferric ions

Impact of *M. whitei* crude root extract on *in vivo* antioxidant potency was investigated by measuring the ferric ions reduction capacity of liver tissue and blood plasma using the FRAP assay. The findings are outlined in Figure 4.5.

In the normal control category, the antioxidant potential in liver tissue was lower than that of diabetic category, with absorbance value of 0.17 ± 0.01 at 700 nm. In the diabetic

control category, the antioxidant capacity in liver tissue was lower than that of groups under treatment, with absorbance value of 0.19 ± 0.02 at 700 nm. Treatment with *M. whitei* at 200 mg/Kg bwt resulted in an 89.5% increase in antioxidant capacity to 0.36 ± 0.04 absorbance units ($p < 0.05$), while the 400 mg/Kg bwt dose resulted in a 200.0% increase to 0.57 ± 0.05 absorbance units ($p < 0.05$). Metformin (100 mg/Kg bwt) exhibited the greatest improvement, with a 300.0% increase in antioxidant capacity to 0.76 ± 0.05 absorbance units ($p < 0.05$), suggesting that while *M. whitei* is effective in restoring antioxidant capacity, metformin remains more potent.

Normal control (0.80 ± 0.04) exhibited lower antioxidant capacity in blood plasma than in the groups under treatment. The diabetic control category conveyed significantly reduced antioxidant capacity, with an absorbance value of 0.68 ± 0.03 at 700 nm, compared to both the normal control group and treatment groups. Treatment with *M. whitei* at 200 mg/Kg bwt moderately increased antioxidant capacity by 17.6% to 0.80 ± 0.04 absorbance units, while the 400 mg/Kg bwt dose further improved antioxidant capacity by 27.9% to 0.87 ± 0.05 absorbance units ($p < 0.05$) suggesting that extract effect was dose/concentration-dependent in enhancing systemic antioxidant defenses. On the other hand, metformin demonstrated a superior effect, increasing antioxidant capacity by 41.2% to 0.96 ± 0.05 absorbance units ($p < 0.05$), showing that metformin's ability to enhance systemic antioxidant defenses is slightly stronger than that of *M. whitei*.

Student's t-test analysis indicated significant differences in antioxidant capacity between the treatment groups and controls for both liver tissue and blood plasma ($p < 0.05$).

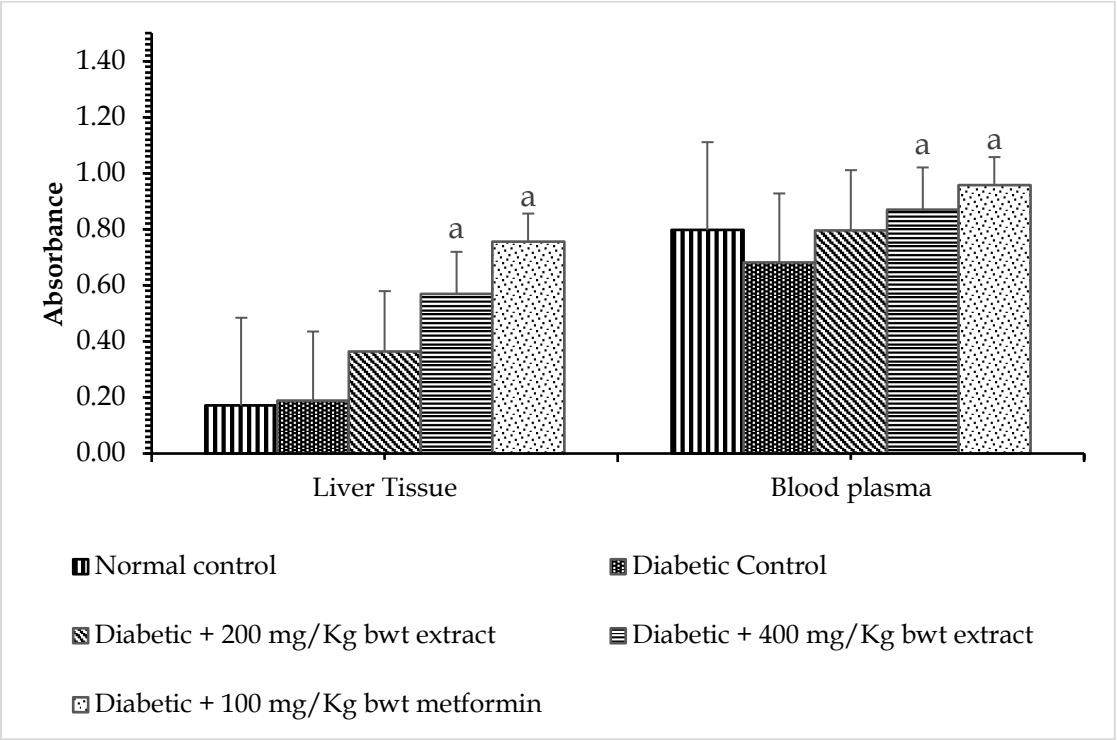

Figure 0.5: Effects of *M. whitei* crude root extract on liver tissue and blood plasma capacity to reduce ferric ions. FRAP assay in liver tissue and blood plasma. Values are presented as mean ± SEM, with n = 6. Bar graphs with letter a are statistically significant compared to diabetic and normal control. ($p < 0.05$, Student's t-test).

4.8 Effects of *M. whitei* crude root extract on lipid peroxidation in liver tissues

Lipid peroxidation in liver tissues was assessed by measuring malondialdehyde (MDA) levels whose absorbance was taken at 532nm, a common marker of oxidative stress. The findings are outlined in Figure 4.6.

Normal control exhibited lower MDA levels (1.58 ± 0.08 µM) compared to the diabetic groups. The diabetic control category demonstrated significantly elevated levels of MDA (2.89 ± 0.12 µM) indicating increased lipid peroxidation and oxidative stress in the liver compared to the treatment groups. Treatment with *M. whitei* at 200 mg/Kg bwt led to a 18.7% reduction in MDA levels, decreasing to 2.35 ± 0.10 µM ($p < 0.05$). The

400 mg/Kg bwt dose of *M. whitei* resulted in a 45.0% reduction in MDA levels, with levels dropping to 1.59 ± 0.09 µM ($p < 0.05$), suggesting a strong dose-dependent protective effect against lipid peroxidation. In comparison, metformin (100 mg/Kg bwt) was equally effective in reducing lipid peroxidation, achieving a 44.3% reduction in MDA levels (1.61 ± 0.07 µM, $p < 0.05$). This suggests that the antioxidant properties of *M. whitei*, particularly at higher doses, are comparable to metformin in mitigating oxidative stress. Student's t test confirmed that the reductions in MDA levels noted in the *M. whitei* treatment groups were significant statistically when contrasted to the diabetic control category ($p < 0.05$).

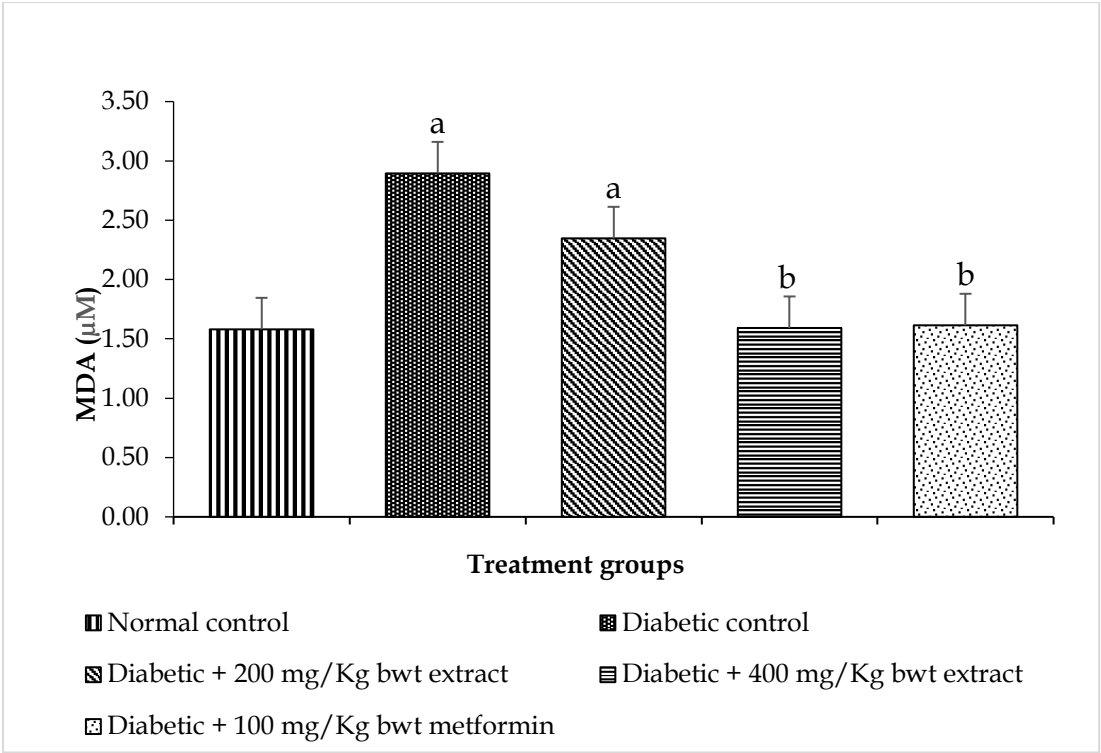

Figure 0.6: Effects of crude *M. whitei* root extract on lipid peroxidation in liver tissues.

Values represent mean ± SEM; n =6. Mean values with b letters on bar graphs are significant statistically compared to diabetic control while those with letter a are

significantly different compared to normal control ($p < 0.05$, Student's t test). MDA-Malondialdehyde.

CHAPTER FIVE

DISCUSSION

This study aimed to assess the qualitative phytochemical composition of *M. whitei* root extract, evaluate its antioxidant properties the *in vitro* state, examine its effects on FBS, FBW, serum lipids, liver and kidney functions serum indices, liver lipid peroxidation, and *in vivo* antioxidant capacity in diabetic Wistar rats. In this study, phytochemical analysis of *M. whitei* root extracts showed the presence of various bioactive compounds which varied across aqueous, hexane, and methanolic solvents. Saponins, phenols, flavonoids, tannins, alkaloids, glycosides, coumarins, steroids/sterols, and terpenoids were detected in both methanol as well as aqueous extract, all the aforementioned phytochemicals were detected except alkaloids. In hexane extract flavonoids and alkaloids were not detected. The qualitative phytochemical variation in extracts acquire from the various solvent indicates that solvent type selection is paramount in extracting specific phytochemicals from *M. whitei* roots. The ability of methanol and aqueous to extract a wider range of phytochemicals can be attributed to their polarity property, which makes them effectively dissolve both polar and semi-polar compounds while hexane being non-polar extracts lesser compounds. This phytochemical analysis of *M. whitei* root extracts aligns with the findings of (Khanal et al., 2022) where methanol and aqueous extracts of *Beilschmiedia roxburghiana* picked up a broader spectrum of phytochemicals than hexane extraction.

This study also explored to analyze *M. whitei* root extracts' *in vitro* antioxidant potential. In the first analysis, DPPH assay was done. DPPH is a compound that consists of a nitrogen-free radical which can be reduced into a non-radical form (DPPH-H) by

a proton radical scavenger or hydrogen-donating antioxidants. DPPH radical exhibits absorbance at 517 nm, which disappears after being transformed to a stable molecule after accepting hydrogen atom or an electron from an antioxidant compound . Methanol as well as aqueous extracts of *M. whitei* roots illustrated antioxidant activity which was dependent on extract concentration. The percentage of DPPH scavenged rose as the extract concentration was elevated, as demonstrated by the absorbance values measured at 517 nm. The methanol extract exhibited a stronger scavenging activity potency at all concentrations compared to aqueous extract, highlighting that methanol solvent was more effective in obtaining compounds with potency to scavenge free radicals as compared to water solvent.

A pivotal hallmark of an agent antioxidant potency is its IC_{50} value, where small values indicate a more percentage of scavenged DPPH radicals and ultimately stronger antioxidant potency. According to (Moriasi et al., 2020) samples whose IC_{50} values is lower than 50 µg/mL are classified as very strong antioxidants, those whose IC_{50} figures ranges between 50 and 100 µg/mL as strong antioxidants, values between 101 and 150 µg/mL as moderate antioxidants, and values above 150 µg/mL as weak antioxidants. In this research study, L-ascorbic acid (the positive control) exhibited the largest percentage of scavenged DPPH radical, an observation further reinforced by its small IC_{50} magnitude of 23.00 µg/mL, therefore proving the most effective antioxidant compound.

Methanol crude extract based on its IC_{50} magnitude of 42.71 µg /mL can be considered as a very strong antioxidant though not to the extent of ascorbic acid. Aqueous extract can be considered as a moderate antioxidant as it exhibited the highest IC_{50} magnitude

of 116.9 µg /mL. Data analysis based on ANOVA revealed differences that were significant ($p< 0.05$) between the *M. whitei* extracts and ascorbic acid at various concentrations, benchmarking disparities in the analyte antioxidant potential. Aqueous and methanol extract exhibited antioxidant properties, though in varying measures, with the methanol extract coming out stronger than aqueous. The potency of methanol extract was closer to that of ascorbic acid. The findings from these assays highlight that methanolic extract of *M. whitei* crude roots extract has natural antioxidant potency with the concentration-dependent increase in DPPH scavenging indicating that higher concentrations of these extracts could be beneficial in applications where antioxidant activity is desired and hence can be considered in developing therapeutics or dietary supplements. The other *in vitro* antioxidant analysis method employed was the FRAP assay which is based on the ability of the phytochemicals (flavonoids, saponin, phenols and tannis) to reduce the ferric ion ($Fe^{3+)}$ to ferrous ion ($Fe^{2+)}$ which can then be examined by absorbance at 700 nm and increases in absorbance at this wavelength indicate an increase in reducing power (Guchu et al., 2020). FRAP assay results in this study demonstrated that methanolic and aqueous extracts of *M. whitei* roots have the capability to reduce ferric ions which increases in a concentration-dependent manner. L-ascorbic acid (the positive control) exhibited significantly higher absorbance values across all concentrations, indicating its higher reducing power compared to both extracts. The IC_{50} value of ascorbic acid (0.23 mg/mL) was significantly lower compared to methanol extract (24.13 mg/mL) and aqueous extract (29.5 mg/mL) further confirming its superior antioxidant capacity. However, the methanol extract exhibited slightly better-reducing power values across all concentrations than the aqueous extract, an observation which was supported by its lower IC_{50} value. This *in vitro* antioxidant

analysis outcome showed that *M. whitei* extracts had radical scavenging potency which can be attributed to it having compounds capable of electron transfer and donating hydrogen (Khanal et al., 2022). This antioxidant potential can be attached to *M. whitei* vast phytochemicals that were found present in this research study (Saponins, phenols, tannins, alkaloids, flavonoids, glycosides, coumarins, steroids/sterols, and terpenoids) which may contain reductive and oxidative capacities that allow absorption and counteracting effects of free radicals (Muthoni Guchu et al., 2020). The antioxidant activity observed in *M. whitei* root extracts using DPPH scavenging and FRAP assay aligns with findings from studies conducted by (Moriasi et al., 2020, Alali et al., 2007, Tyagi et al., 2010) where methanol and aqueous extracts of *Piliostigma thonningii*, Jordan plants and *Flacourtica indica* exhibited antioxidant properties and also agree with the studies conducted by (Alali et al., 2007, Tyagi et al., 2010) which reported that methanolic extracts of plants displayed a stronger antioxidant potency compared to their aqueous counterparts.

This study also examined the effect of *M. whitei* on fasting blood sugar (FBS) levels in STZ-induced diabetic rats. STZ is the furthermost used diabetogenic chemical for creating rat models of T1DM and T2DM (Ghasemi & Jeddi, 2023, Lenzen, 2008). The two components of STZ molecule are the nitrosourea group, which causes a particular, quick, and irreversible cytotoxic activity that destroys pancreatic β-cells, and the glucopyranosyl group, which helps glucose transporter II (GLUT2) absorb it (Ghasemi & Jeddi, 2023). Experiments have provided evidence that the main drive for STZ-induced β-cells death is DNA alkylation a property that is attributed to its nitrosourea moiety, more so at the O^6 guanine position (Szkudelski, 2001) which facilitates the transfer of the methyl group from streptozotocin to the DNA molecule causing damage

which results in the fragmentation of the DNA and in the attempt to repair DNA, poly (ADP-ribose) polymerase (PARP) is overstimulated (Szkudelski, 2001). This diminishes cellular NAD+, and subsequently ATP stores. The depletion of the cellular energy stores ultimately results in beta-cell necrosis (Szkudelski, 2001). Streptozotocin also methylates proteins, which is likely to contribute to the functional defects of the beta cells after exposure to streptozotocin (Lenzen, 2008,). In this study, after inducing diabetes with STZ, the diabetic rats displayed elevated FBS levels compared to the normal control group, thus confirming the hyperglycemic state. Treatment with *M. whitei* root extract at both 200 mg/Kg and 400 mg/Kg body weight significantly reduced FBS levels over the 21 days. The efficacy of *M. whitei* extract was dose-dependent where the higher dose (400 mg/kg) achieved a more pronounced effect which approached the hypoglycemic effectiveness of metformin, the standard anti-diabetic drug utilized as positive control. FBS levels continued to elevate significantly in the diabetic control group during the study period reflecting the hyperglycemia progression if treatment is unavailable. The rats in the normal control category displayed stable FBS levels within the normal range during the whole study period, reaffirming the normal FBS levels in a healthy physiological state. The metformin-treated group exhibited the most pronounced decrease in FBS levels while the 400 mg/kg *M. whitei* extract group showed a slightly lesser reduction, indicating that though its efficacy was slightly reduced than metformin's, it still provided a significant therapeutic effect. These results show that the *M. whitei* extract possesses antihyperglycemic properties capable of mitigating the hyperglycemia induced by STZ. Therefore *M. whitei* extract may possess bioactive compounds capable of mimicking or complementing the action of standard hypoglycemic agents. The extract's ability to reduce FBS levels to a range approaching

that of metformin is notable, especially considering that metformin widely used and considered the most appropriate therapy for patients with type-two diabetes mellitus (Sanchez-Rangel & Inzucchi, 2017). The hypoglycemic properties of *M. whitei* can be linked to its rich phytochemical content as evidenced in this research study, which includes flavonoids, saponins, phenols and tannins. These bioactive molecules have been found to have antioxidants, anti-inflammatory, immunomodulatory, hypolipidemic, and anti-hyperglycemic activities (Yedjou et al., 2023). According to (Yedjou et al., 2023), hypoglycemic herbs like *M. whitei* increase insulin secretion, enhance glucose intake by adipose and muscle tissues, inhibit glucose absorption from the intestine, and suppress hepatic glucose production. According to (Kumar et al., 2023), flavonoids exhibit significant antioxidant and hypoglycemic effects. These compounds can attenuate tissue damage caused by prolonged hyperglycemia by increasing endogenous antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) while reducing ROS (Gandhi et al., 2020). This antioxidant bioactivity helps protect pancreatic β-cells from oxidative stress, thereby improving insulin secretion and action. Flavonoids are also known to modulate various molecular mechanisms, including the prevention of high glucose-induced cell proliferation, thereby protecting against diabetes-related complications (Gandhi et al., 2020). This study concurs with the study done by (Ravi et al., 2004) that the presence of these phytochemicals in *M. whitei* could enhance its efficacy in protecting tissue defense systems against oxidative damage, similar to how flavonoids strengthen the antidiabetic potential of other plants like *Eugenia jambolana* (Ravi et al., 2004). Phytosterols, another key component found in *M. whitei*, exhibit high antioxidant activity and makes significant contribution to hypoglycemic and hypolipidemic effects

(Kumar et al., 2023). These compounds are capable of modulating lipid metabolism, which is commonly disrupted in diabetic conditions, thereby helping to reduce blood glucose levels. The multifaceted action of these phytochemicals suggests that *M. whitei* could exert its hypoglycemic effects through pancreatic mechanisms which may involve extensive β-cells regeneration (Kim et al., 2007) after the distortion of pancreatic islet cells caused by STZ (Momodu et al., 2014) and stimulation of β-cells, promoting insulin release and also through extra pancreatic actions which could include stimulation of peripheral glucose utilization, enhancement of glycolysis and glycogenesis, and inhibition of intestinal glucose absorption, thereby reducing blood glucose levels (Rawi et al., 2011). This study also agrees with the study done by (Rawi et al., 2011) which reports that extract of *Psidium guajava* showed hypoglycemic effects by stimulating glucose uptake by peripheral tissues and inhibiting endogenous glucose production as maybe the case in *M. whitei* extract. The hypoglycemic effects observed with *M. whitei* are consistent with findings in other medicinal plants traditionally used for diabetes management. Appreciable studies have indicated that various plant extracts containing bioactive molecules like flavonoids, saponins, alkaloids, and phenolic acids exhibit antidiabetic properties through various mechanisms, including enhancement of secretion of insulin, boosting sensitivity of insulin, and exerting antioxidant effects. For example, (Ravi et al., 2004, Kiptisia et al., 2020, Koech ets al., 2020, Yedjou *et al., 2023)* demonstrated that phytochemical-rich plant extracts significantly lowered the levels of sugars in the blood of diabetic models, similar to the results seen with *M. whitei*.

Furthermore, *M. whitei* crude root extract also impacted body weight in STZ-induced diabetic rats over the 21-day treatment period, with comparisons made to non-diabetic

controls and metformin-treated groups. These findings shed light on the potential protective effects of *M. whitei* in mitigating weight loss associated with diabetes, albeit not fully preventing it. In the normal control category, the body weights of the rats increased by 7% over the 21 days and this slight increase reflects normal growth and health which is expected in healthy animals. In contrast, the experimental control cohort with diabetes exhibited a significant weight loss of 15% over the same period. Treatment with *M. whitei* at both 200 mg/Kg and 400 mg/Kg bwt led to reduced body weight loss in comparison to experimental control cohort with diabetes, pointing that *M. whitei* may help mitigate diabetes-related catabolic effects. The 200 mg/Kg bwt extract-treated group showed a 14% reduction in body weight while the 400 mg/Kg bwt group experienced a 13% reduction. While these reductions lack statistical significance in comparison to the non-diabetic control category, their severity was lower than the weight loss observed in untreated diabetic rats. This indicates that *M. whitei* might possess properties that can partially counteract diabetes-induced weight loss, which might be due to its ability to reduce hyperglycemia which was an indication of proper glucose utilization (Pari & Amarnath Satheesh, 2004), its protective effect in muscle wasting and controlling protein turnover over and/or improvement in diabetes mellitus associated disorders (Oyedemi et al., 2012). The metformin-treated group showed a 13% reduction in weight, which is comparable to the group treated with 400 mg/Kg bwt of *M. whitei*. This suggests that *M. whitei* at higher doses may have a comparable effect to metformin in reducing weight loss in diabetic animals, although neither treatment prevented weight loss entirely. The weight loss observed in this research study corresponds with findings in other studies, such as, (Ravi et al., 2004) who reported decreased body weight in diabetic rats due to excessive breakdown of tissue

proteins and (King, 2012) who highlighted that in chemically induced diabetic models, a big portion of pancreatic beta cells are destroyed, which causes reduction in insulin production, promoting to hyperglycemia and weight loss.

This study also revealed the significant modulatory impact of *M. whitei* crude root extract on serum lipid profiles in diabetic rats, notably affecting triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). These findings suggest that *M. whitei* may serve as a prospective therapeutic agent in managing dyslipidemia associated with diabetes. Elevated TG levels in the control cohort with diabetic are consistent with the dyslipidemia commonly seen in diabetes, characterized by increased TG synthesis and decreased TG clearance due to insulin resistance (Ciumărnean et al., 2020; Singh et al., 2013). High dosage treatment of *M. whitei* (400 mg/Kg bwt) caused a 59.3 % reduction in TG levels, a reduction that was greater than the one caused by metformin treatment. This reduction effect may be attributed to the presence of flavonoids and saponins which works by regulating Acetyl-CoA carboxylase and fatty acid synthase enzyme action and as a consequence inhibit synthesis of TG (Das et al., 2018). Additionally, flavonoids lowers TG levels by modulating various lipid metabolism (Suanarunsawat et al., 2016). Peculiarly, treatment with 200 mg/Kg bwt dose of *M. whitei* yielded an increase in TG levels, which was unexpected, nevertheless this could be indicative of a dose-dependent response, a case which has also been observed in other studies involving medicinal plants (Stohs & Ray, 2015). It has been documented that low dosages of some phytochemical compounds may be unable to activate pathways leading to lipid modulation (Sreelatha & Inbavalli, 2012). The 14% reduction in total cholesterol that yielded as a result of treatment with *M. whitei* is closely related to the

13.2% reduction yielded by treatment with metformin. This suggests that *M. whitei* has significant potential for managing hypercholesterolemia. The ability of *M. whitei* to lower cholesterol levels is likely due to saponins presence, which act by binding to bile acids, reducing cholesterol absorption in the intestine, ultimately enhancing cholesterol excretion (Suanarunsawat et al., 2016). Flavonoids have as well been linked to cholesterol levels reduction an impact brought by as a result of elevating bile acid excretion and blocking cholesterol absorption in the guts, this promotes cholesterol excretion as well (Ansari et al., 2023; Ciumărnean et al., 2020). *M. whitei* exhibits a comparable potency in modulating total cholesterol levels when compared to other medicinal plants such as *Curcuma longa* and *Aloe vera* which have cholesterol modulating effects as well (Alkandahri et al., 2024). These among other plants have been largely explored for their potenctial to down regulate cholesterol by acting on earlier mentioned metabolic pathways, underscoring the increasing interest in healthy natural therapies for lipid modulation. The 52.6% significant increase in HDL-C conveyed by the group under the 200 mg/Kg bwt treatment dosage of *M. whitei* suggests that the extract not only reduces harmful lipids but also promotes reverse cholesterol transport, a critical process for cardiovascular health. Flavonoids have been shown to upregulate liver X receptors (LXRs), leading to enhanced HDL-C synthesis and reduced cardiovascular risk, which is particularly important for diabetic patients (Ciumărnean et al., 2020; Gandhi et al., 2020b). This increase in HDL-C is promising, given that higher HDL-C levels are linked to with a reduced risk of cardiovascular complications in diabetes (Das et al., 2018). It is noteworthy that the higher dose of 400 mg/Kg bwt did not further increase HDL-C levels, suggesting a possible saturation point, beyond which no additional benefits are observed. Similar dose-saturation effects

have been reported in studies of other plant-based therapies, emphasizing the need for optimal dosing strategies (Stohs & Ray, 2015). The moderate reduction in LDL-C (10.26%) observed with *M. whitei* at 400 mg/Kg bwt, while less potent than the 28.2% reduction achieved with metformin, still represents a favorable outcome. LDL-C, or "bad cholesterol," is a major contributor to the development of atherosclerosis, and its reduction is a key target in managing cardiovascular risk in diabetic patients (Ahmed et al., 2023; Moke et al., 2023). The LDL-C-lowering effect observed in this study can be linked to the flavonoids and saponins presence in *M. whitei*, which are known to increase LDL receptor expression and enhance bile acid excretion, thereby lowering circulating LDL-C levels (Ansari et al., 2023; Das et al., 2018; Nugroho et al., 2012). Although *M. whitei*'s effect on LDL-C is less pronounced compared to metformin, its overall impact on multiple lipid parameters, particularly the significant increase in HDL-C, suggests that it offers a broader and more holistic approach to managing dyslipidemia. This multi-faceted effect, targeting not just LDL-C but also TG and HDL-C, may provide additional cardiovascular benefits in diabetic patients (Singh et al., 2013; Suanarunsawat et al., 2016).

Additionally, the crude root extract of *Mondia whitei* demonstrated significant improvements in various serum biochemical markers of liver and kidney function in STZ-induced diabetic rats. Treatment with *M. whitei* resulted in dose-dependent reductions in kidney function serum indices namely urea and creatinine, as well as liver enzymes (ALT, GGT, ALP) and enhancements of total protein and albumin levels which were indicators of liver function. Although the higher dose of *M. whitei* (400 mg/Kg bwt) approached the efficacy of metformin for many of these parameters, metformin exhibited superior overall effects, particularly in reducing liver enzyme

levels and improving kidney function. In the diabetic control group, elevated levels of urea and creatinine indicated impaired kidney function, likely due to diabetic nephropathy. Treatment with *M. whitei* led to a 14.4% reduction in urea at 200 mg/Kg bwt and a 4.2% reduction at 400 mg/Kg bwt. Additionally, creatinine levels were reduced by 19.7% at the 400 mg/Kg bwt dose. Though these reductions were insignificant, *M. whitei* effects on kidney function serum indices can be attributed to its potential to reduce stress caused by oxidative and inflammation in kidney tissues (Putra et al., 2023; Arabshomali et al., 2023). These results are consistent with previous research on how medicinal plants' phytochemicals can prevent diabetic nephropathy (Thilagavathi et al., 2023). However, metformin produced more substantial reductions in urea (19.8%) and creatinine (25.4%), demonstrating greater efficacy in mitigating kidney dysfunction. Metformin's known mechanisms, including improving insulin sensitivity, reducing hyperglycemia, and lowering oxidative stress, likely contribute to its superior nephroprotective effects (Moke et al., 2023; Zhu et al., 2023). The elevated liver enzyme levels (ALT, AST, GGT, ALP) in the experimental diabetic control category indicate significant liver damage, likely resulting from chronic hyperglycemia and damage caused by oxidation (Scarpa et al., 2024). Treatment with *M. whitei* at 400 mg/Kg bwt reduced ALT by 22.7% and AST by 23.9%, indicating hepatoprotective effects. These findings are in agreement with the antioxidant properties of *M. whitei*, which likely neutralize the radicals which are free and prevent peroxidation of lipids in liver tissues (Putra et al., 2023). However, metformin demonstrated superior effects, reducing ALT by 31.5% and AST by 43.2%, highlighting its stronger hepatoprotective properties. Metformin's mechanisms include activating AMPK, reducing hepatic glucose output, and improving lipid metabolism, which explains its pronounced effects

on liver enzyme profiles (Dutta et al., 2023; Foretz et al., 2023). Interestingly, *M. whitei* at 400 mg/Kg bwt also reduced GGT by 20.8% and ALP by 33.0%, which is comparable to metformin's reductions of 32.3% in GGT and 27.9% in ALP. This suggests that while *M. whitei* is less potent than metformin, it shows promise in improving liver function by reducing oxidative stress and enhancing protein metabolism. Decreases in total protein and albumin levels in the experimental diabetic control category group indicated impaired liver protein synthesis. Treatment with *M. whitei* at 200 mg/Kg bwt resulted in an 8.5% increase in total protein and a 7.9% increase in albumin, while the 400 mg/Kg bwt dose led to increases of 10.2% in total protein and 9.9% in albumin. These results suggest that *M. whitei* improves liver protein synthesis, potentially by safeguarding liver cells from ROS induced damage and promoting normal liver function (Ansari et al., 2023). In comparison, metformin exhibited greater efficacy, increasing total protein by 13.2% and albumin by 12.7%, probably due to its effects on reducing inflammation of hepatic tissue as well as oxidative strains (Zhang et al., 2022). These findings indicate that *M. whitei* has the potential to complement metformin therapy by providing additional hepatoprotective benefits, particularly in restoring protein metabolism. The observed hepatoprotective effects of *M. whitei* agree with prior studies on plant-based therapies rich in flavonoids, phenolic compounds, and saponins, which have been found to mitigate ROS induced stress and inflammation while improving metabolic profiles in diabetic models (Ahmed et al., 2023; Thilagavathi et al., 2023). Flavonoids, in particular, have been recognized for their ability to inhibit lipid peroxidation, scavenge free radicals, and protect liver and kidney cells from injury (Kamiloglu et al., 2024; Scarpa et al., 2024). Studies on similar medicinal plants, such as *Moringa oleifera* and *Vernonia amygdalina*, have

demonstrated comparable improvements in liver and kidney function, further supporting the potency of *M. whitei* as an antioxidant and anti-inflammatory (Ansari et al., 2023; Dissa Ayu Putri, 2024.). The stronger effects observed with metformin correlate with its well-documented ability to activate AMPK, reduce hepatic gluconeogenesis, and improve lipid metabolism (Jin & Arroo, 2023). Metformin's capacity to lower liver enzyme levels, enhance insulin sensitivity, and mitigate oxidative stress has been established in animal models and clinical settings (Arabshomali et al., 2023; Jin & Arroo, 2023). The phytochemicals present in *M. whitei*, including flavonoids, tannins, saponins, and phenolic compounds, are likely linked to the extract's beneficial effects on liver function. Flavonoids and phenolic compounds have been recognized for their ability to reduce ROS induced stress by scavenging ROS agents and inhibiting lipid peroxidation, thus preventing damage of cells in the liver and kidneys (Arabshomali et al., 2023; Putra et al., 2023). Additionally, the saponins in *M. whitei* may enhance lipid metabolism and promote protein synthesis, further contributing to its protective effects on liver function. The antioxidant activity of *M. whitei* observed in this study aligns with previous research on plant-based antioxidants. The improvements in liver enzyme levels and protein synthesis suggest that *M. whitei* protects hepatocytes from oxidative damage and may help restore normal liver function. In contrast, metformin employs its effects through multiple pathways, including AMPK activation, which promotes lipid oxidation, reduces glucose production in the liver, and improves mitochondrial function (Foretz et al., 2023; Zhu et al., 2023). This multifaceted mechanism likely explains metformin's superior effects on liver function compared to *M. whitei*. These findings suggest that *M. whitei* crude root extract may serve as a complementary therapy for improving liver function in diabetic patients.

While *M. whitei* is not as potent as metformin, its dose-dependent effects indicate that it could be used alongside conventional therapies to enhance therapeutic outcomes. The ability of *M. whitei* to reduce key markers of liver damage highlights its potential to prevent or mitigate diabetes-induced organ damage.

The antioxidant capacity of *M. whitei* crude root extract was also further investigated *in vivo* using the FRAP assay, measuring ferric ion reduction in both liver tissue and blood plasma. The findings indicated that *M. whitei* plays a significant role in restoring antioxidant capacity in diabetic conditions, although metformin exhibited a more pronounced effect overall. In the diabetic control group, the antioxidant capacity in liver tissue was significantly reduced, reflecting the oxidative stress regularly linked with diabetes (Ciumărnean et al., 2020; Moke et al., 2023). This oxidative stress arises from an imbalance between the production of ROS and the body's antioxidant defenses, contributing to cellular damage and metabolic dysfunction (Ahmed et al., 2023; Ansari et al., 2023). The FRAP assay results demonstrated a marked reduction in antioxidant capacity in the diabetic group (0.19 ± 0.02 absorbance units), align with existing studies that links diabetes to compromised antioxidant status in the liver (Scarpa et al., 2024). Treatment with *M. whitei* crude root extract led to the significant restoration of antioxidant capacity in a dose-dependent manner. The 200 mg/Kg bwt dose resulted in an impressive 89.5% increase in antioxidant capacity, while the 400 mg/Kg bwt dose yielded a remarkable 200% increase, underscoring the extract's potential in enhancing hepatic defenses against oxidative damage. This dose-dependent response suggests that higher concentrations of bioactive compounds in *M. whitei* may effectively stimulate both enzymatic and non-enzymatic antioxidant systems, such as superoxide dismutase and glutathione peroxidase (Arabshomali et al., 2023; Stohs & Ray, 2015). However,

while *M. whitei* demonstrated significant improvements, metformin outperformed the extract, increasing antioxidant capacity by 294.7%. Metformin's mechanisms include modulation of mitochondrial function and enhanced redox balance, which are essential for mitigating oxidative stress (Singh et al., 2013). A similar trend was witnessed in the blood plasma antioxidant capacity, where the experimental diabetic control category demonstrated significantly lower ranges (0.68 ± 0.03 absorbance units) in comparison to the normal control group (0.80 ± 0.04). This systemic reduction in antioxidant defenses highlights the extensive oxidative stress present in diabetes, affecting not only local tissue but also circulating antioxidant levels (Ansari et al., 2023; Gandhi et al., 2020b). Treatment with *M. whitei* at 200 mg/Kg bwt resulted in a moderate increase of 16.2% in plasma antioxidant capacity, while the 400 mg/Kg bwt dose further improved this by 27.9%, indicating that *M. whitei* can enhance systemic antioxidant defenses in a dose-dependent manner. Although these increases are encouraging, metformin again showed superior efficacy, enhancing plasma antioxidant capacity by 39.7%, thereby reinforcing its role as a potent antioxidant agent (Ciumărnean et al., 2020). ANOVA analysis confirmed significant differences between the treatment categories, with post hoc comparisons using Tukey's HSD test indicating that the improvements observed in the *M. whitei* treatment groups were statistically significant compared to the diabetic control group ($p < 0.05$). These results clearly establish that *M. whitei* exerts beneficial effects on antioxidant capacity in both liver tissue and blood plasma, although its efficacy is somewhat limited compared to pharmaceutical agents like metformin. The ability of *M. whitei* to restore antioxidant capacity may be primarily attributed to its abundant composition of flavonoids, saponins, and other bioactive compounds recognized for their antioxidant properties. Flavonoids, particularly, are distinguished

for their ability to scavenge free radicals and upregulate endogenous antioxidant systems, effectively reducing oxidative damage (Das et al., 2018.). In a diabetic state, ROS are normally elevated, and the oxidative stress that comes as a consequence of this fosters the development of diabetes and its complications, this state presents the phytochemicals with a good platform to exert their antioxidant properties (Idaguko & Adeniyi, 2023, Moke et al., 2023).

In this study, treatment impact of *M. whitei* crude root extract on protection of liver tissue against lipid peroxidation was analyzed. This was attained by checking the levels of MDA, a biomarker of lipid peroxidation. On the normal control group, very minimal levels of MDA were detected, this reflection of a normal physiological state, where there is minimal oxidated stress being induced by ROS. In the diabetic control group, significantly high levels of MDA were registered which was an indication of the great measure of lipid peroxidation. This can be linked to the absence of treatment which could have otherwise alleviated oxidative stress induced by ROS. Lipid peroxidation is an autocatalytic destructive mechanism that involves oxidation unsaturated fatty acids of the liver, yielding lipid hydroperoxides and MDA, this process is in great measure mediated by reactive oxygen species (Merghem et al., 2019; Patro et al., 2016). MDA is an aldehyde compound (electrophile species) and it is prone to various reactions, this makes it interact with vast molecules resulting to various complications such as neurodegenerative disorders, genotoxic, toxic stress and exert cytotoxicity of cellular molecules. MDA is greatly utilized as a biomarker to monitor the levels oxidative stress induced by reactive oxygen species (Merghem et al., 2019). Thio barbituric acid (TBA) assay has widely been exploited and is still the most utilized assay (even to date) in determining the extent lipid peroxidation in an organism (Merghem et al., 2019). TBA

assay relies on the formation of a red adduct as a consequence of the interaction of MDA with TBA (Garcia et al., 2005), this adduct can be quantified by a spectrophotometer at 530 nm (Merghem et al., 2019). Elevated blood glucose levels lead to overproduction of ROS, this ROS contributes immensely to oxidative stress and therefore bring about damage of cells, tissue and organs. Treatment with *M. whitei* reduced MDA ranges in a dose-dependent manner, indicating that the extract possesses antioxidant potency that can mitigate oxidative damage. In the clutch treated with 200 mg/Kg bwt extract MDA levels decreased by 18.7% while in 400 mg/Kg bwt dosage group MDA levels decreased by 45.0%. This significant decrease indicates a strong dose-dependent protective effect of *M. whitei* against oxidative stress, probably due to an increased concentration of antioxidant compounds. The ability of *M. whitei* to markedly lower MDA levels indicate its potency to substantially reduce lipid peroxidation and mitigate liver damage. Metformin treatment reduced MDA levels by 44.3%, a reduction that is similar to that observed in the 400 mg/kg dose of *M. whitei* suggesting that *M. whitei*, particularly at higher doses, is nearly as effective as metformin in reducing oxidative stress and protecting liver tissue from lipid peroxidation. The findings in this study are consistent with (Merghem et al., 2019) study which illustrated MDA levels were decreased by methanol extract of *Ruta montana,* this study as well agrees with the proposal that bioactive molecules contributes to antioxidant activities and protection of liver tissues as has been highlighted in the existing literature since *M. whitei* is rich with such molecules. Hybrid action of various bioactive molecules present in *M. whitei,* such as terpenoids, flavonoids, alkaloids, phenols, tannins, and saponins can be associated to antioxidant and hepatoprotection displayed by it . Bioactive molecules have vital contribution in

alleviating ROS induced stress by enacting their antioxidant properties in protecting cells and tissues against ROS related damage and death . Flavonoids and phenolic molecules have been well recognized for their potency to neutralize free radicals by terminating the chain reaction of ROS . Studies have related strongly the concentration of phenols in an analyte to the antioxidant potency of that analyte this suggests that phenols might be the major promoters of antioxidant potency displayed by various plant extracts and in this particular study *M. whitei*. Polyphenols are appreciably effective antioxidants, and are centrally involved in protection of cells and tissues against ROS induced damage and ROS induced stress associated complications such as degenerative disorders (Adefegha & Oboh, 2012). Bioactive molecules are capable of exerting their antioxidant effect by acting through several mechanisms such as neutralizing free radicals, ligating metal ions, enhancing antioxidant enzymes as well as reduction of ROS. Phenolic and flavonoid compounds inhibit lipid peroxidation, indicating that a plant extract with this bioactive molecules can serve as natural antioxidant agent by protecting tissues against peroxidation of free fatty acids as well as protecting cells and tissue form ROS degradation (Yüksel et al., 2021). The ability of *M. whitei* to mitigate lipid peroxidation of liver tissues, further illuminated by the complementary findings from both the *in vivo* and the *in vitro* antioxidant studies provides compelling evidence of its antioxidant properties which can largely be strongly associated to its vast phytochemicals.

One limitation of this research study is that crude extracts which contain a wide mixture of phytochemicals were used and therefore individual compounds responsible for any observed bioactivity have not been identified. Additionally, while aqueous and methanol extracts contain more phytochemicals, the study does not yet establish the

concentration or potency of these compounds, which can significantly impact their antioxidant efficacy. Future research could involve fractionation and quantitative analysis of *M. whitei* root extract to pinpoint and quantify the most bioactive components.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This research study has shown that *M. whitei* crude root extract has saponins, phenols, tannins, alkaloids, flavonoids, glycosides, coumarins, steroids/sterols, and terpenoids with absence of anthraquinones from the qualitative phytochemical analysis performed. Also, *in vitro* antioxidant studies showed that *M. whitei* root extract possess significant antioxidant activity, as demonstrated by their concentration-dependent scavenging of DPPH radicals and ferric ions (Fe^{3+}) reducing power.

This study also found that administering the methanolic extract of *M. whitei* to STZ-induced diabetic Wistar rats resulted in a dose-dependent reduction in fasting blood sugar (FBS) levels, with the higher dose (400 mg/Kg body weight) approaching the efficacy of metformin, a standard anti-diabetic drug in mitigating hyperglycemia as well as partially attenuating diabetic induced weight loss rate. Additionally, *M. whitei* extract significantly modulated serum lipid profiles, reducing triglycerides (TG) and total cholesterol (TC), while notably increasing high-density lipoprotein cholesterol (HDL-C) in diabetic rats, which collectively highlights its potential to address dyslipidemia in diabetic conditions. Furthermore, this study revealed that *M. whitei* provided hepatoprotective effects in diabetic rats, evidenced by improved liver enzyme levels (ALT, GGT, ALP). These protective effects are likely due to the rich phytochemical composition of *M. whitei* extract, particularly flavonoids, saponins, and phenolic compounds, known for their antioxidant and anti-inflammatory properties. On the other hand, *M. whitei* did not significantly reduce urea and creatinine levels, which are serum indices of kidney function.

Additionally, *in vivo* antioxidant studies further demonstrated that *M. whitei* crude root extract significantly enhanced the antioxidant capacity of both liver tissue and blood plasma in STZ-induced diabetic rats as evidenced by the reduction of ferric ions (Fe^{3+}). This antioxidant activity was dose-dependent, with the 400 mg/Kg body weight extract dose exerting a substantial improvement in liver tissue antioxidant potency. The antioxidant potential of *M. whitei* root extract was further confirmed by the lipid peroxidation studies where malondialdehyde (an index of lipid peroxidation) levels in liver tissues of diabetic groups treated with *Mondia whitei* crude root extract significantly reduced in comparison to the diabetic untreated control group.

Taken together, the phytochemical-rich extract of *M. whitei* crude root extract showed antidiabetic, antioxidant, lipid modulation, and hepatoprotective effects in STZ-diabetic induced rats. The findings from this research study underscore the therapeutic potential of *Mondia whitei* crude root extract as a complementary therapy in managing diabetes mellitus and its associated complications. Its multifaceted effects on glycemic control, lipid modulation, and tissue protection in diabetic condition make it a valuable addition to alternative complementary diabetes mellitus management approaches.

6.2 Recommendations

From this study, the following recommendations are put forth;

- *M. whitei* root could be utilized as an alternative herbal remedy in managing DM and its complications as it has shown;
  - ✓ Antidiabetic efficacy in STZ-induced diabetic Wistar rats
  - ✓ Significant antioxidant potency

- To further understand the bioactivity of *M. whitei* root extract, subsequent studies could focus on isolating specific bioactive compounds and examining individual therapeutic action.
- Given the diverse qualitative phytochemical profile of *M. whitei* root extract, evaluating these extracts for other pharmacological activities, such as anti-inflammatory or antimicrobial properties, besides conducting studies on the other parts of *M. whitei* should be considered for future investigation.
- Since *M. whitei* root extract demonstrated a dose-dependent effect, long-term toxicity studies are recommended to ensure its safety at higher doses over extended treatment periods. This will be vital for supporting its potential viability as a long-term treatment option.

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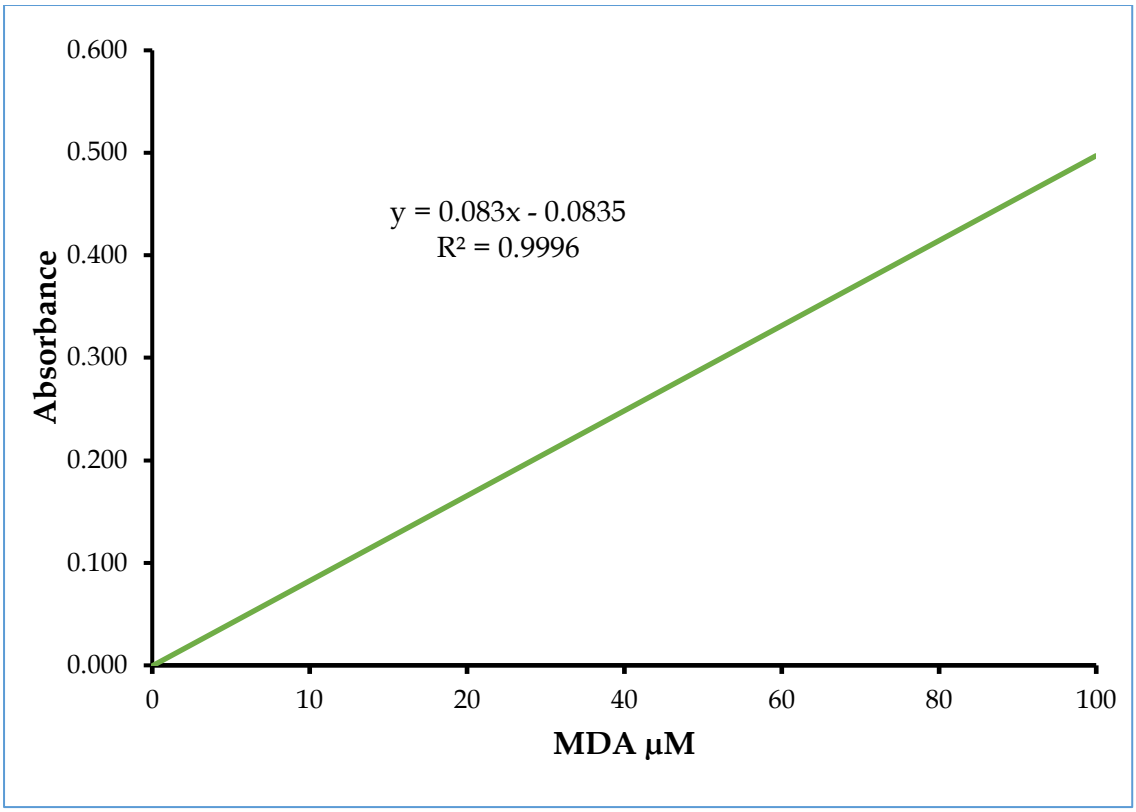
5 APPENDICES

Appendix I: Standard Calibration Curve for Malondialdehyde (MDA) Analysis



Appendix II: NACOSTI Research License

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Date of Issue: 24/February/2024

RESEARCH LICENSE

This is to Certify that Mr.. kennedy kamau Gitau of University of Eldoret, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Uasin-Gishu on the topic: ANTIOXIDANT AND ANTIDIABETIC PROPERTIES OF Mondia whitei ROOT EXTRACT IN STREPTOZOTOCIN-INDUCED DIABETIC WISTAR RATS for the period ending : 24/February/2025.

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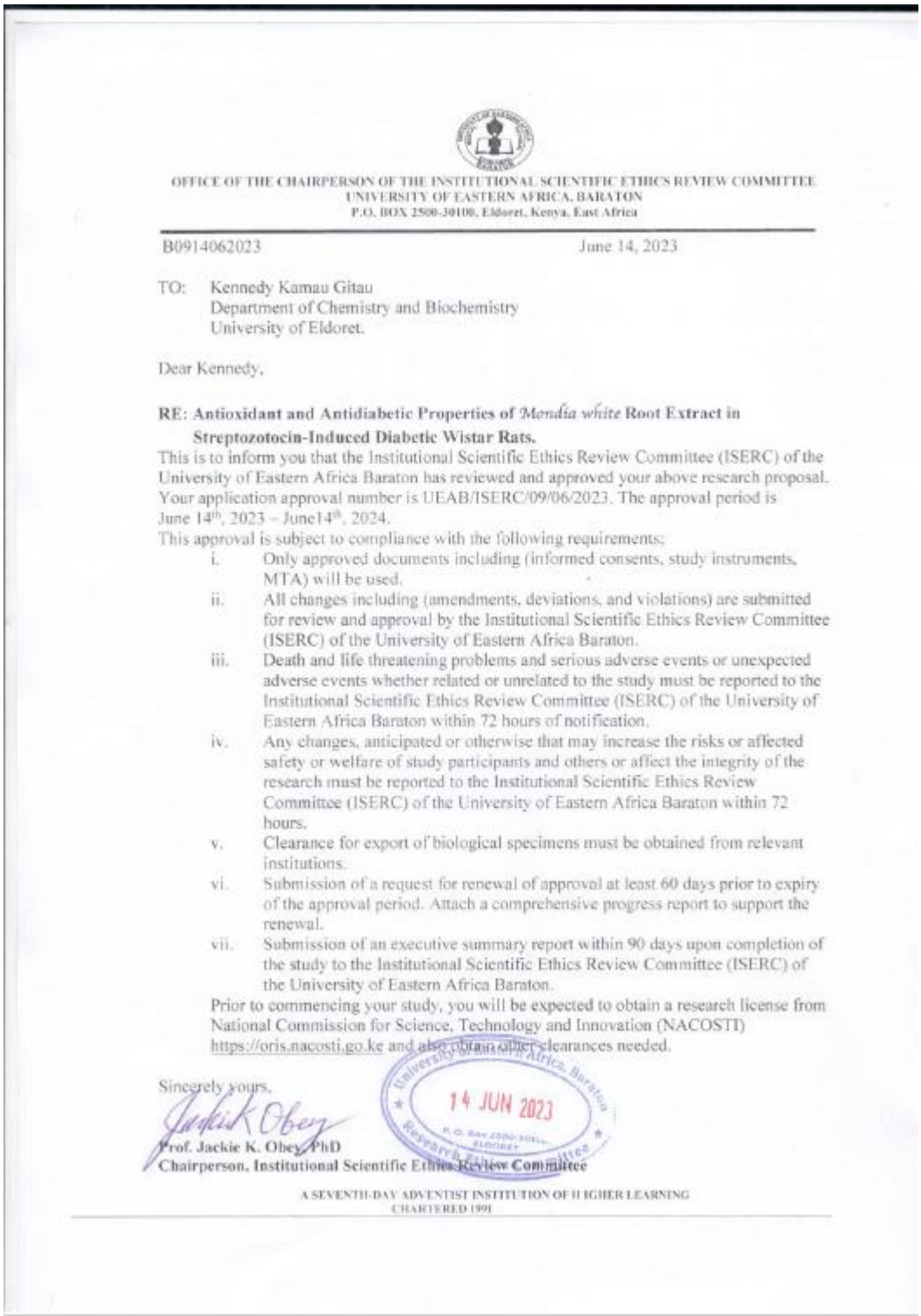
Appendix III: Ethical Clearance Letter



OFFICE OF THE CHAIRPERSON OF THE INSTITUTIONAL SCIENTIFIC ETHICS REVIEW COMMITTEE
UNIVERSITY OF EASTERN AFRICA, BARATON
P.O. BOX 2500-30100, Eldoret, Kenya, East Africa

B0914062023 June 14, 2023

TO: Kennedy Kamau Gitau
Department of Chemistry and Biochemistry
University of Eldoret.

Dear Kennedy,

RE: Antioxidant and Antidiabetic Properties of *Mondia white* Root Extract in Streptozotocin-Induced Diabetic Wistar Rats.

This is to inform you that the Institutional Scientific Ethics Review Committee (ISERC) of the University of Eastern Africa Baraton has reviewed and approved your above research proposal. Your application approval number is UEAB/ISERC/09/06/2023. The approval period is June 14th, 2023 – June14th, 2024.

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by the Institutional Scientific Ethics Review Committee (ISERC) of the University of Eastern Africa Baraton.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to the Institutional Scientific Ethics Review Committee (ISERC) of the University of Eastern Africa Baraton within 72 hours of notification.
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to the Institutional Scientific Ethics Review Committee (ISERC) of the University of Eastern Africa Baraton within 72 hours.
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to the Institutional Scientific Ethics Review Committee (ISERC) of the University of Eastern Africa Baraton.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

Sincerely yours,

Jackie K. Obey

Prof. Jackie K. Obey, PhD
Chairperson, Institutional Scientific Ethics Review Committee

14 JUN 2023

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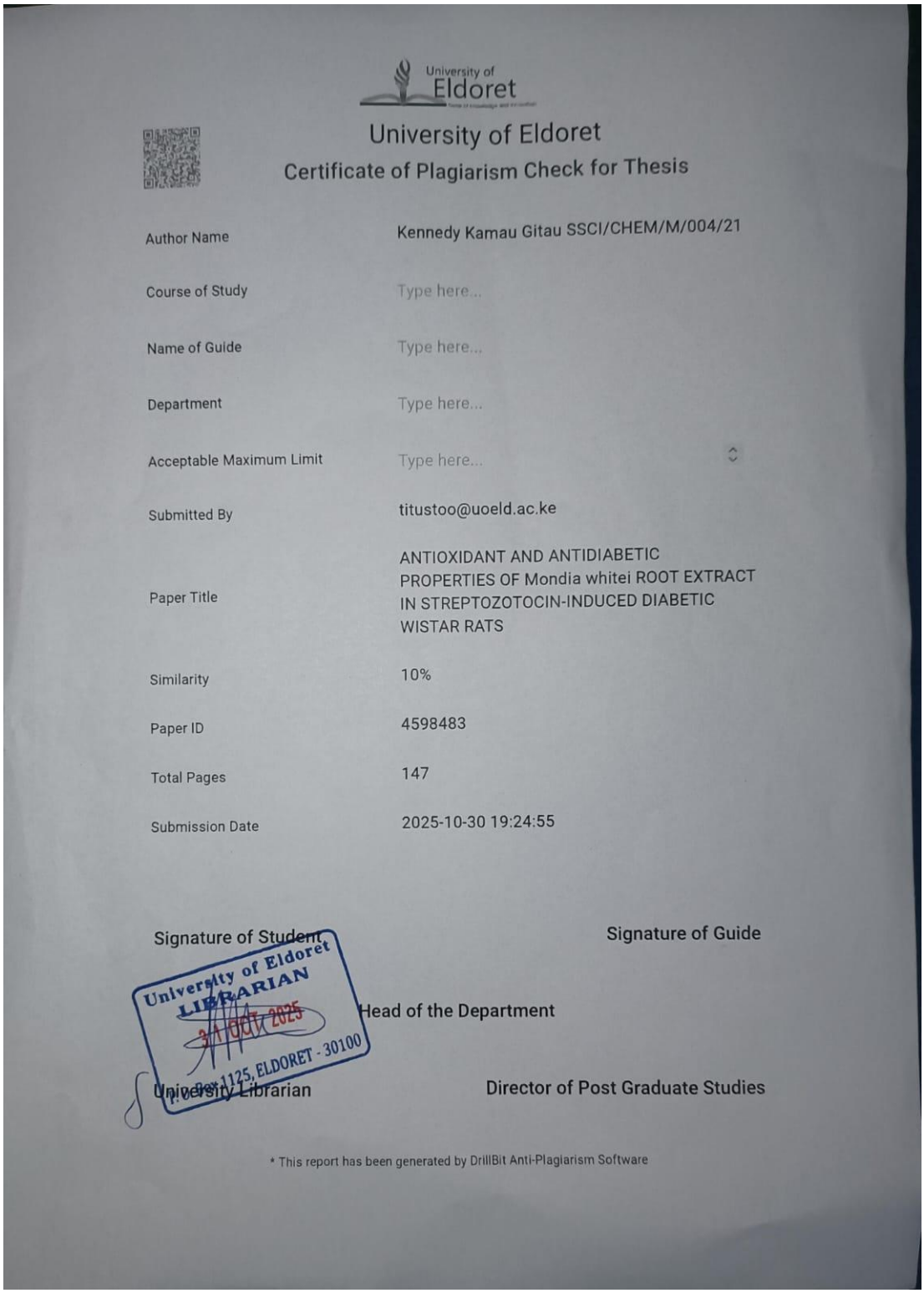
Appendix IV: Similarity Report



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