ANTIDIABETIC PROPERTIES OF *Tarchonanthus camphoratus* IN FRUCTOSE-INDUCED DIABETIC WISTAR RATS

BY

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DECLARATION

Declaration by the candidate

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

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DEDICATION

This thesis is dedicated to my dear wife Grace and to my loving children; Peace, Lawi and Chiri for their daily commitment and unending inspiration throughout my research period. God bless you.

ABSTRACT

Tarchonantus camphoratus (TC) has been used traditionally to manage several diseases such as diabetes mellitus (DM) including in Kenya but its anti-diabetic efficacy has not been scientifically evaluated. Therefore, this study aimed at evaluating the antidiabetic properties of *Tarchonantus camphoratus* crude leaf extract in fructose-induced diabetic Wistar rats. DM in rats was induced using high fructose (25% w/v) in drinking water in experimental groups for 12 weeks. Rats were divided after the DM induction into five groups (n=7 per group) as follows: Group I, normal control; Group II, diabetic untreated; Group III diabetic treated with metformin (100 mg/kg.bw/day), Groups IV and V; diabetic treated with 300 (low dose –LD) and 600 (high dose – HD) mg/kg.bw/day of TC extract respectively. Oral treatments were administered daily for 21 days. Changes in fasting body weights and blood glucose levels were monitored weekly. At the end of the treatment period, oral glucose tolerance test, skeletal muscle tissue weights and serum lipid profile parameters were analysed. For renal function, serum creatinine and urea were analysed while for liver function, serum alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT), total proteins (TP), C-reactive protein (CRP) and albumin (ALB) were analysed. The skeletal muscle triglyceride (TG) mass was also analysed. Phytochemicals in the TC crude leaf extract were qualitatively analysed using standard procedures. Statistical analysis was done by Tukey's test and one-way analysis of variance (ANOVA). Values with $p < 0.05$ were considered to be statistically significant. After 12 weeks, DM was successfully induced in the diabetic untreated group with rats having significantly higher body weights compared to all other groups ($p < 0.05$). As compared to the untreated controls, there was a significant amelioration in fasting hyperglycemia in HD and LD groups (33.9% and 27.30% respectively). There was also increased glucose tolerance observed in both treatment groups. Further, TC extract significantly improved fructose-induced hypertriglyceridemia in the treatment groups compared with DM groups. The serum levels of ALP, ALT, and CRP were significantly reduced while TP and ALB were elevated in the extract-treated diabetic rats compared with unaltered DM rats. DM group also exhibited significantly higher skeletal muscle TG mass when compared to normal control and diabetic treatment groups. The observed hypoglycaemic and hypolipidemic activities in the diabetic treatment groups could be associated with the phytochemicals present in TC extract. TC crude leaf extract therefore possesses potential for alternative medicine for DM treatment and management.

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LIST OF ABBREVIATIONS

ADA	American Diabetes Association
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BP	Blood pressure
CVD	Cardiovascular disease
DHAP	Dihydroxyacetone phosphate
DKA	Diabetic ketoacidosis
DM	Diabetes mellitus
DPP-IV	Dipeptidyl peptidase 4
FBG	Fasting blood glucose
FFA	Free fatty acids
G6P	Glucose-6-phosphate
G6Pase	Glucose-6-phosphatase
GAD	Glutamic acid decarboxylase
GDM	Gestational diabetes mellitus
GIP	Glucose-dependent insulinotropic peptide
GLP-1	Glucagon-like peptide
GLUT4	Glucose transporter type 4
GSK-3	Glycogen synthase kinase 3
HbA1c	Glycated hemoglobin
HDL	High density lipoproteins
HF	High fructose

HLA	Human leukocyte antigen
HPL	Human placental antigen
IAAs	Anti insulin antibodies
ICCA	Islet cell cytoplasmic antibodies
ICSA	Islet cell surface antibodies
IDDM	Insulin dependent diabetes mellitus
IDF	International Diabetes Federation
IGT	Impaired glucose tolerance
IR	Insulin resistance
IRS	Insulin receptor substrate
LDL	Low density lipoproteins
LMICs	Low middle income countries
MHC	Major Histocompatibility Complex
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
NCD	Non-communicable diseases
NIDDM	Noninsulin dependent diabetes mellitus
OADs	Oral antidiabetic drugs
OGTT	Oral glucose tolerance test
PEPCK	Phosphoenolpyruvate carboxykinase
PPARγ	Peroxisome proliferator-activated receptor gamma
SD	Standard deviation
SEM	Standard error of mean

SUR-1   Sulfonylurea receptor

SUs   Sulfonylureas

T1DM   Type 1 diabetes mellitus

T2DM   Type two diabetes mellitus

TC   *Tarchonanthus camphoratus*

TF   Transcription factor

TG   Triglycerides

TZDs   Thiazolidinediones

VLDL   Very low density lipoprotein

WHO   World Health Organization

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May God bless you all!

CHAPTER ONE

INTRODUCTION

1.1 Background information

Chronic hyperglycemia, often known as Diabetes mellitus (DM), is a metabolic condition caused by anomalies in the metabolism of carbohydrate, fat and protein due to decreased insulin secretion, insulin action or both (Vieira *et al.*, 2019). Type 2 diabetes mellitus (T2DM), type 1 diabetes mellitus (T1DM), and gestational diabetes (GD) are the three different kinds of diabetes mellitus. It is challenging to keep blood glucose levels within the desired range when you have T2DM, a progressive, lifelong condition caused by the body's inadequate use of insulin (Galicia-Garcia *et al.*, 2020). Elevated blood glucose levels can cause complications such as blindness, cardiovascular disease, renal failure, peripheral neuropathy, lower-extremity amputations and erectile dysfunction (Verhulst *et al.*, 2019). Consequently, there is a necessity for proper glycemic control among patients with type 2 diabetes so as to reduce the risk of diabetes-related complications. Fortunately, good diabetes care and management can prevent or retard the onset of these complications. Type 1 diabetes mellitus occurs in childhood and is primarily because of autoimmune-mediated destruction of pancreatic beta cells generating absolute insulin deficiency(Décio L Eizirik *et al.*, 2020). Gestational diabetes is a temporary condition that occurs during pregnancy and involves an increased risk of developing diabetes and long-term complications for both mother and child (Ilonen *et al.*, 2019; McIntyre *et al.*, 2019).

Worldwide, more than 3.2 million people died as a result of hyperglycemia in 2017. Around 80% of the total deaths among diabetics occurred in low-income and middle-income countries (LMICs) (Awuchi *et al.*, 2020; WHO, 2014). Both the prevalence and incidence of type 2 diabetes are increasing worldwide, particularly in developing countries, in conjunction with increased obesity rates and westernization of lifestyle and diet (Blüher, 2019; Hu, 2011). In Kenya, the adult population nationally adjusted prevalence of diabetes mellitus was estimated to be 3.1% in 2019 and is projected to rise to 4.4% in 2035 if mitigations are not put in place to address this rise (Mwai *et al.*, 2021). More than 8,700 diabetes-related deaths were registered in Kenya in 2015, almost all under 60 years of age (Mwai *et al.*, 2021). This rise in diabetes mellitus is associated with demographic and social changes such as globalization, urbanization, aging population, adoption of sedentary lifestyles and consumption of unhealthy diets (Mwai *et al.*, 2021; Sun *et al.*, 2022). The WHO has predicted that the major burden of DM will occur in developing countries. Life expectancy may be halved by diabetes mellitus especially in LMICs where its prevalence is increasing and treatment is often unavailable. It is also projected that diabetes mellitus will be the seventh leading cause of death in the world by 2030 (Khan *et al.*, 2020). The attendant economic burden for health care systems is skyrocketing, owing to the costs associated with low productivity, treatment and diabetes complications. T2DM remains a leading cause of cardiovascular disorders, blindness, end-stage renal failure, amputations, and hospitalizations (Taylor *et al.*, 2021). It is also associated with increased risk of cancer, serious psychiatric illness, cognitive decline, chronic liver disease, accelerated arthritis, and other disabling or deadly conditions

(Taylor *et al.*, 2021). Therefore, it is crystal clear that effective DM management strategies are of great importance.

Management of T1DM requires use of exogenous insulin whereas T2DM requires oral medication but may also need insulin, blood pressure control and foot care at later stages (Savitha Subramanian *et al.*, 2021). There are three main classes of oral hypoglycemic drugs; the sulphonylureas (e.g glimepiride, glipizide, gliclazide and glibenclamide), biguanides (e.g metformin), and dipeptidyl peptidase-4 inhibitors (DPP-4 Inhibitors) e.g sitagliptin, saxagliptin, alogliptin, and vildagliptin. Other classes are sodium-glucose cotransporters-2 (SGLT2) inhibitors, glucagon-like peptide 1 receptor agonists (GLP-1RA), glinides, thiazolidinediones and α-glucosidase inhibitors (Dahlén *et al.*, 2022) . These diabetes drugs are often inaccessible or too costly and insulin for example needs a cold chain for preservation. Furthermore, diabetes drugs have numerous side effects that include drug induced hypoglycemia, pancreatitis and hepatotoxicity (Haq *et al.*, 2021)

On the other hand, herbal plants have been used by traditional health practitioners for management of diabetes mellitus (Kasole *et al.*, 2019; van Huyssteen *et al.*, 2011). Plants with antidiabetic properties are important for the development of economically viable and effective treatment of the disease. One such plant with promising potential for use in the treatment of diabetes is *T. camphoratus* (TC). *Tarchonanthus camphoratus* is widely distributed in a variety of habitats, including thickets of Masai Mara, grassland, forest and semi-desert. Additionally, it is a plant of uplands in Kenya mostly growing in natural environment of altitudes ranging from 1,000 to 3,000 meters, common plant of the savanna biome, dry forest margins or secondary deciduous bushland, woodland and

wooded grassland often dominant or co-dominant and commonly associated with Acacia spp (Happold *et al.*, 2013).

Traditional health practitioners from the Kipsigis community in Kenya use TC for management of diabetes. TC is known to contain many compounds including saponins (Aro *et al.*, 2021; Mukherjee *et al.*, 2006), flavanons and tannins (Nasr *et al.*, 2020) that are potentially hypoglycemic. Also, previous *in vitro* studies of aqueous and ethanolic extracts of TC on Chang liver cells and C2C12 muscle cells respectively showed effectiveness in glucose utilization in concentration-independent trends (Cock *et al.*, 2021). Despite this good anti-diabetic potential, the anti-diabetic efficacy of TC aqueous extracts has not been shown. This study is thus designed to determine the antidiabetic effects and efficacy of *T.camphoratus* crude leaf extracts in fructose-induced diabetic Wistar rats.

1.2 Statement of the problem

The rapid increase in DM prevalence and incidence is strongly related to changes in lifestyle including reduced physical activity and unhealthy diets. Consumption of diets rich in sugars such as fructose or the fructose- containing sugar, sucrose, commonly used as sweeteners in soft drinks and pastries is closely linked to the development of metabolic syndromes such as T2DM, dyslipidemia and obesity (Dupas *et al.*, 2017). The result is increasing economic burden for the families affected and health care systems owing to the treatment, productivity loss and caregivers' costs associated with diabetes and its complications. Additionally, diabetes drugs are often inaccessible or too expensive and extra costs of hospital admissions. Furthermore, diabetes drugs have numerous side

effects which include; weight gain, drug induced hypoglycemia, pancreatitis, liver toxicity, lactic acidosis and gastrointestinal upsets (Haq *et al.*, 2021) . In the light of these challenges, focus is now shifting towards the use of natural products for pharmacological purposes. Many published reports and journals show that some plants crude extracts possess antidiabetic activity (Haq *et al.*, 2021; Nirmala *et al.*, 2009), yet they show fewer side effects and are cheaper compared to usual conventional antidiabetic agents (Sohn *et al.*, 2010; Tran *et al.*, 2020).

1.3 Justification of the study

Fructose consumption has increased dramatically in recent years incorporated in industrial products and soft drinks. This has led to increased rates of obesity, insulin resistance, and metabolic syndrome and is clearly multifactorial due to physical inactivity and unhealthy eating habits. The hallmarks of the surge in obesity prevalence is as a result increased consumption of fructose emanating from dietary sugars, primarily sucrose and high-fructose corn syrup (Taş, 2020). Mostly, increased fructose intake from sucrose or high fructose corn syrup has been implicated in promoting weight gain, visceral adiposity, dyslipidemia, and insulin resistance, which are all components of the metabolic syndrome associated with T2DM (Mortera *et al.*, 2019). Therefore it is important to develop a suitable model that mimics the diet similar to actual human consumption. *T.Camphoratus* leaves in this study was based on the claims that it is has continued to be used traditionally as remedy for DM management. Wistar rats were used because they are complex and heterogeneous as the human, readily available, and cost-effective animal model that can be replicated with ease without facing reproducibility issues (Okoduwa *et al.*, 2017). *Tarchonantus Camphoratus* is used by traditional herbalist

from the Kipsigis communities as a boiled concoction to treat diabetes. However, no scientific studies have been done to prove its antidiabetic activity the hence current study. The conventional drugs used for treatment of diabetes are costly, and with adverse side effects. Attention therefore needs to be directed to alternative forms of medicines of plant origin since they are locally available, cheaper and have minimal side effects compared to conventional medicine. There is enough evidence that TC reduces blood glucose levels and can be used for management of DM; however, *in vivo* studies need to be conducted to determine its efficacy.

The present study will therefore use diet-induced diabetic animal models to investigate the efficacy and antidiabetic properties of *T.camphoratus* aqueous leaf extract.

1.4 Study objectives

1.4.1 Overall objective

The overall objective of this study was to determine the antidiabetic properties and efficacy of *T. camphoratus* (TC) aqueous crude leaf extract in fructose-induced diabetic Wistar male rats.

1.4.2 Specific objectives

The specific objectives of the study are:

I. To qualitatively determine the phytochemical components of *Tarchonanthus camphoratus* aqueous leaf extracts.

II. To determine the effects of *Tarchonanthus camphoratus* leaf extracts on skeletal muscle tissue & body weights, blood glucose and glucose tolerance in fructose-induced diabetic Wistar rats.

III. To investigate whether *Tarchonanthus camphoratus* leaf extracts ameliorates skeletal muscle adiposity and hyperlipidemia in fructose-induced diabetic Wistar rats.

IV. To determine the effects of *Tarchonanthus camphoratus* leaf extracts on serum indices of liver & kidney functions in fructose-induced diabetic Wistar rats.

1.5 Research questions

i. What phytochemical components are present in *Tarchonanthus camphoratus* aqueous crude leaf extract?

ii. How does *Tarchonanthus camphoratus* leaf extracts affect skeletal muscle tissue and body weight, blood glucose and glucose tolerance of high fructose-induced diabetic Wistar rat model?

iii. Does *Tarchonanthus camphoratus* leaf extracts ameliorate skeletal muscle adiposity and hyperlipidemia in fructose-induced diabetic Wistar rats?

iv. How does TC leaf extracts affect serum markers of liver and kidney function?

1.6 Overall significance of the study

The study might provide scientific data on the antidiabetic efficacy of TC that will be useful to traditional health practitioners and also for plausible development of economically viable natural plant derived drugs with fewer side effects. Also the use of the rat model provide insights into better understanding of hyperglycemia and its complications and where TC works best in the management of specific metabolic syndromes. The outcome of study is expected to impact positively by reducing economic

burden and complications associated with available conventional synthetic drugs used to manage diabetes mellitus.

CHAPTER TWO

LITERATURE REVIEW

2.1 Diabetes mellitus

Diabetes mellitus (DM) is defined as a heterogeneous metabolic disorder of glucose management (Perveen *et al.*, 2018) caused by different factors characterized by a chronic high level of blood sugar (Balaji *et al.*, 2019) with disturbances to carbohydrate, fat, and protein metabolism (Ozder, 2014) resulting from defects in insulin secretion, insulin action, or both (Poznyak *et al.*, 2020). DM reduces the individuals body's ability to regulate the level of glucose in the blood stream resulting in a number of major and some minor complications (Nazir *et al.*, 2018).

The chronic hyperglycemia of diabetes mellitus (DM) is associated with end organ damage, dysfunction, and failure in organs and tissues including the retina, kidney, nerves, heart, and blood vessels. The International Diabetes Federation (IDF) estimated an overall prevalence of diabetes mellitus to be 366 million in 2011, and in 2019, it was estimated that 463 million people had diabetes and this is projected to rise to 578 million by 2030 clearly indicating diabetes mellitus as one of the fastest growing global health emergencies of the 21st century.

2.2 Classification of diabetes mellitus

The 1980 WHO Expert Committee proposed two major classes of diabetes mellitus that was widely accepted and globally adopted. These classifications were named insulin dependent diabetes mellitus (IDDM), or type 1; and non-insulin dependent diabetes mellitus (NIDDM), or type 2 (WHO, 2019). In the 1985 Study Group Report the terms

type 1 and type 2 were omitted but IDDM and NIDDM were retained, and a new class of malnutrition-related diabetes mellitus (MRDM) introduced. The 1985 WHO classification was principally based on clinical descriptions, with specific focus on the pharmacologic patient management. Both reports from WHO recognized other types of diabetes mellitus that included gestational diabetes mellitus (GDM) as well as impaired glucose tolerance (IGT) (Sreenivasamurthy, 2021).

Scientists have divided diabetes mellitus into three different types:

2.2.1 Type 1 diabetes mellitus (T1DM)

Type 1 diabetes mellitus is caused by an autoimmune reaction in which the body's immune system attacks the insulin-producing beta cells of the pancreatic islet. Resultantly, the body makes very little or no insulin. The causes of this detrimental process are not fully understood but a likely implicated cause is that the combination of genetic susceptibility conferred by multiple genes including human leukocyte antigen (HLA) genotypes and an environmental trigger, such as a viral infection, toxins or diet, initiate the T cell-mediated autoimmune reaction (Awuchi *et al.*, 2020). The HLA region on chromosome 6p21 accounts for approximately 50% of the familial aggregation of T1DM and its association with T1DM has been known for over 40 years. The strongest association is with HLA DR and DQ. HLA DR and DQ are cell surface receptors that present antigens to T-lymphocytes. Both DR and DQ are alpha-beta heterodimers. The DR alpha chain is encoded by the DRA locus, and the DR beta chain is encoded by DRB loci. Similarly, DQA1 and DQB1 loci encode the alpha and beta chains, respectively, of the DQ molecule. The DR and DQ loci are highly linked to each other and, to a lesser degree, to other HLA loci (Redondo *et al.*, 2018). The condition can develop at any age,

although it occurs most frequently in children and young people, a significant proportion is often diagnosed during adulthood. The latent autoimmune diabetes of adults (the LADA) is a diagnostic term used when the type 1 diabetes develops in adults; and it has a slower onset than same condition in children. T1DM is one of the most common chronic diseases in childhood, and is becoming more common due to the increase in childhood overweight and obesity (Awuchi *et al.*, 2020; Leslie *et al.*, 2016). Type 1 diabetes mellitus or insulin dependent diabetes mellitus (IDDM) is also known as juvenile onset diabetes. It accounts for 5-10% of total cases and is characterized by autoimmune or idiopathic beta-cell destruction, subsequently leading to development of absolute insulin deficiency (Hare *et al.*, 2022).

2.2.2 Gestational diabetes mellitus

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset or first recognition during pregnancy, due to placental hormones that promotes insulin resistance. GDM is diagnosed for the first time during pregnancy and may occur anytime during pregnancy. It happens in about 2 to 10% of all pregnancies and may disappear or improve after delivery (transient condition). However, after pregnancy about 5 to 10% of women with gestational diabetes mellitus are found to have diabetes mellitus, most commonly type 2 diabetes (Dipla *et al.*, 2021; Szmuilowicz *et al.*, 2019). GDM develops in women with insufficient insulin secretory ability to overcome the decreased action of insulin (insulin resistance) due to hormone production by the placenta. Risk factors for GDM include older age, overweight and obesity (a body mass index, BMI, of greater than 30), previous GDM, excessive weight gain during pregnancy (Nair *et al.*, 2021; Yong *et al.*, 2020).

During pregnancy, placental hormones, such as human placental lactogen and placental growth hormone, cause a gradual development of insulin resistance, which elevates maternal blood glucose levels in order to supply the developing fetus with the adequate amount of glucose. Consequently, the number of maternal β-cells increases, and insulin synthesis and secretion increase in parallel (Stern *et al.*, 2021). In women with GDM, the raised insulin resistance, together with a relative inability of the pancreatic β-cells to adapt to the growing needs, are implied as the main pathophysiological events bringing about glucose intolerance and hyperglycemia (Banerjee, 2018). Complications of GDM include preeclampsia, neonatal macrosomia, neonatal hypoglycemia, large for gestational age offspring and a heightened risk for caesarean delivery. Women with a personal history of GDM also have an enhanced risk for developing T2DM later in life. Regular exercise is crucial for a healthy pregnancy and can downgrade the risk of developing GDM (Johns *et al.*, 2018)

2.2.3 Type 2 diabetes mellitus (T2DM)

Type 2 diabetes mellitus is a metabolic disease characterized by a fasting hyperglycemia frequently progressive and related to development of resistance towards insulin as well as reduced glucose tolerance (T2DM). The imperfect responsiveness of the body tissues to insulin is thought to involve the insulin receptors. However, the specific defects are idiopathic. It is also referred as noninsulin dependent diabetes mellitus (NIDDM), or adult-onset diabetes (Petersen *et al.*, 2018; Sylow *et al.*, 2021). Worldwide, T2DM is the most prevalent and accounts for 90 –95% of all patients with diabetes mellitus (Kaur *et al.*, 2018; Mekala *et al.*, 2020). Its development is primarily as a result of a combination of two major factors: defective insulin secretion by pancreatic β-cells and the inability of

insulin-sensitive tissues to respond to insulin (Galicia-Garcia *et al.*, 2020). Insulin release and action have to precisely meet the metabolic need; hence, the molecular mechanisms involved in the synthesis and release of insulin as well as the insulin response in tissues must be tightly regulated. Therefore, anomalies in any of the mechanisms involved can cause a metabolic imbalance that leads to the pathogenesis of T2DM (Corkey *et al.*, 2021). In this regard, T2DM is found in individuals who are insulin-resistant and who usually have relative insulin deficiency. Initially and often throughout their lifetime, these individuals do not need insulin treatment to survive (Marín-Peñalver *et al.*, 2016).

There are probably many different causes of this form of diabetes mellitus. Although the specific etiologies of T2DM are not known, autoimmune destruction of pancreatic islets β-cells does not occur. Most patients with this form of diabetes are obese, with obesity itself causing some degree of insulin resistance. Patients who are not obese by traditional weight criteria may have an increased percentage of body fat distributed mostly in the abdominal region In T2DM, adipose tissue ameliorates IR through numerous inflammatory mechanisms, augmented free fatty acid (FFA) release, and adipokine down-regulation. The organs implicated in T2DM development include the pancreas, liver, skeletal muscle, kidneys, brain, small intestine, and adipose tissue that contribute to the glucose homeostasis (Galicia-Garcia *et al.*, 2020; Malone *et al.*, 2019).

Diets with high caloric contents especially high fructose are associated with increasing risk of T2DM and insulin resistance. Fructose corn syrup is used commercially as a sweetening agent instead of glucose or sucrose in the preparation of desserts, condiments, and carbonated beverages. Consumption of high amounts of this refined carbohydrate in food and beverages increases the risk of dyslipidemia, obesity, insulin resistance and

heart disease (Sadowska *et al.*, 2019; Wali *et al.*, 2021). Epidemiological studies in humans confirm the association between diabetes prevalence and sugar availability. In addition, chronic intake of a Western diet, characterized by foods rich in sugar, too much consumption of white rice and abundant in total and saturated fat has been suggested to play a role in the development of T2DM (Lozano *et al.*, 2016; Rippe *et al.*, 2016). Diabetes produces substantial changes in intracellular metabolism in most tissues, including the liver. Insulin resistance and excessive accumulation of lipids is greatly associated with non-alcoholic fatty liver disease (NAFLD), which represents the hepatic manifestation of a systemic impairment of the insulin network. NAFLD outline different and well defined degrees of histological hepatic damages. These modifications range from simple nonalcoholic fatty liver accumulation (with a fat content in the hepatocytes higher than 5%) to nonalcoholic steatohepatitis (NASH) characterized by hepatocyte damage and cell death. Ultimately, the collagen deposition and subsequent vascular remodeling occasions fibrosis, cirrhosis and end-stage liver diseases (Mu *et al.*, 2019). However, large differences have been seen in the dose and duration of fructose consumption to induction of T2DM (de Farias Lelis *et al.*, 2020). This type of diabetes is an intricate and heterogeneous disorder that is distinguished by advanced decline in insulin action or defect in insulin signal transduction. In this case, the tissues can't utilize glucose as usual, that is associated with serum triglycerides enhancement, serum HDL reduction and sometimes LDL enhancement Mengwei Li *et al.* (2022).

2.3 Epidemiology and burden of diabetes mellitus

T2D is a serious socio-economic problem that imposes a heavy burden on both the person suffering from the disease as well as the public health system. Epidemiological studies

have shown that more than 100 million people in the world suffered from T2DM (Sinisterra-Loaiza *et al.*, 2019). The number of patients with T2DM is increasing rapidly in both developed and developing countries around the world. This emerging global pandemic is driven by diabetes risk factors, majorly the rising levels of obesity, sedentary life style and poor diets combined with aging population. Furthermore, cheap availability of high-fat and high-energy food in combination with less physical activity has led to the increasing prevalence of obesity. Obesity can cause impaired glucose tolerance, which can lead to increased susceptibility to diabetes manifestation. However, decreasing mortality among those with the disease can be attributed to improved management (Sun *et al.*, 2022). The global prevalence of T2DM is projected to increase to 7079 individuals per 100,000 by 2030, reflecting a continued rise across all regions of the world, most of this rising prevalence will be in developing countries (Khan *et al.*, 2020). By 2025 there will be about 192 million women and 188 million men with T2DM with a substantial greater number of them living in urban (179 million) compared with rural areas (81 million) (Maffi *et al.*, 2017). The prevalence of diabetes in adults aged 20–79 years was estimated to be 8.8% in 2015 and is predicted to rise to 10.4% in 2040. Despite the fact that all types of diabetes are on the increase, the number of people with type 2 diabetes mellitus is expected to increase by 55% by 2035 (Ogurtsova *et al.*, 2017; Sun *et al.*, 2022).

In Africa, metabolic syndrome and T2DM is increasingly becoming a major chronic disease and health burden. The number of overweight and obese people is soaring across Africa due to changes in lifestyles and decreased physical activity, more sedentary lifestyles, changing dietary habits and accessible inexpensive processed fast foods

coupled with increasing levels of obesity, cultural habits, and increasing rapid urbanization (Godman *et al.*, 2020). Currently there are 24 million adults (20-79) in Africa Region in 2021with diabetes, mainly T2DM (95%). This figure is estimated to grow to 33 million by 2030 and 55 million people by 2045 if left uncontrolled.

52 million adults (20-79) in Africa Region have Impaired Glucose Tolerance (IGT) which predisposes them to T2DM and is estimated to get to 71 million by 2030 and 117 million by 2045. 13 million (54%) adults living with diabetes in Africa remain undiagnosed. The projected mortality due to diabetes was 416,000 deaths in the Africa continent in the year 2021. Additionally, approximately, 1 in 8 live births in the IDF Africa Region are afflicted by hyperglycaemia during pregnancy.This has a significant effect on morbidity, mortality and costs in the region. There are several issues to address to lessen the impact of T2DM including bettering detection rates and current access to services besides addressing challenges of over-reliance to prescribed medicines. Similarly, high rates of co-morbidities with infectious diseases such as HIV and tuberculosis in patients in Africa with T2DM require attention (Federation, 2021; Mapa-Tassou *et al.*, 2019).

In Kenya, NCD diabetes prevalence in the year 2012 was between 2.7% in the rural and 10% in the urban areas according to reports by IDF 2015 (Atlas, 2015). In the same year, Diabetes Management and Information Centre in Nairobi, Kenya reported an estimated rate of impaired glucose tolerance at 8.8% and 14.4% in rural and urban areas respectively. These figures may be underestimated because of the low level of awareness of diabetes among adults and higher prevalence of pre-diabetes. The low levels of awareness or undiagnosed people living with diabetes probably contribute to low levels

of treatment though its mainly the high cost of treatment associated with diabetes care that contributes to the low treatment levels among those who are aware of their condition (Mohamed *et al.*, 2018).

There is an increasing proportion (59% of the population) of young people under the age of 25 diagnosed with diabetes in Kenya. This trend may be fueled by urbanization and lifestyle changes, such as increased consumption of refined foods and physical inactivity as Kenya transitions economically, epidemiologically and demographically (Mkuu *et al.*, 2019). Stunted growth and starvation in utero and early life among Kenyan children has been associated with the development of diabetes in adulthood. These trends suggest that heightened and sustained life-course approaches to prevent and improve diabetes care at the population level are necessary (Shiroya *et al.*, 2019). Studies in Kenya's largest referral hospital (KNH) reported 30% of patients with diabetic ketoacidosis died within 48 h after presentation, accounting for 8% of diabetic admissions (Shiroya *et al.*, 2019). Such late presentations for care, alongside challenges like shortage of health-care workers and limited knowledge on diabetes management; high cost of medication and inadequate patient follow-up constitute the predicament for patients (Musoma *et al.*, 2020). Other reports on national health facility census in 2013 and a health system assessment in 2017 further exemplified that Kenyan health facilities are largely unprepared to provide NCD services with diabetes being one of them (Shiroya *et al.*, 2019).

Diabetes contributes to a substantial amount of the global health expenditure in the world as a result of the increase in DM global prevalence as shown in Figure 2.1. There is an inadequacy of information on the economic burden of diabetes in Kenya and Africa continent as a whole, with very limited number of studies in the area.
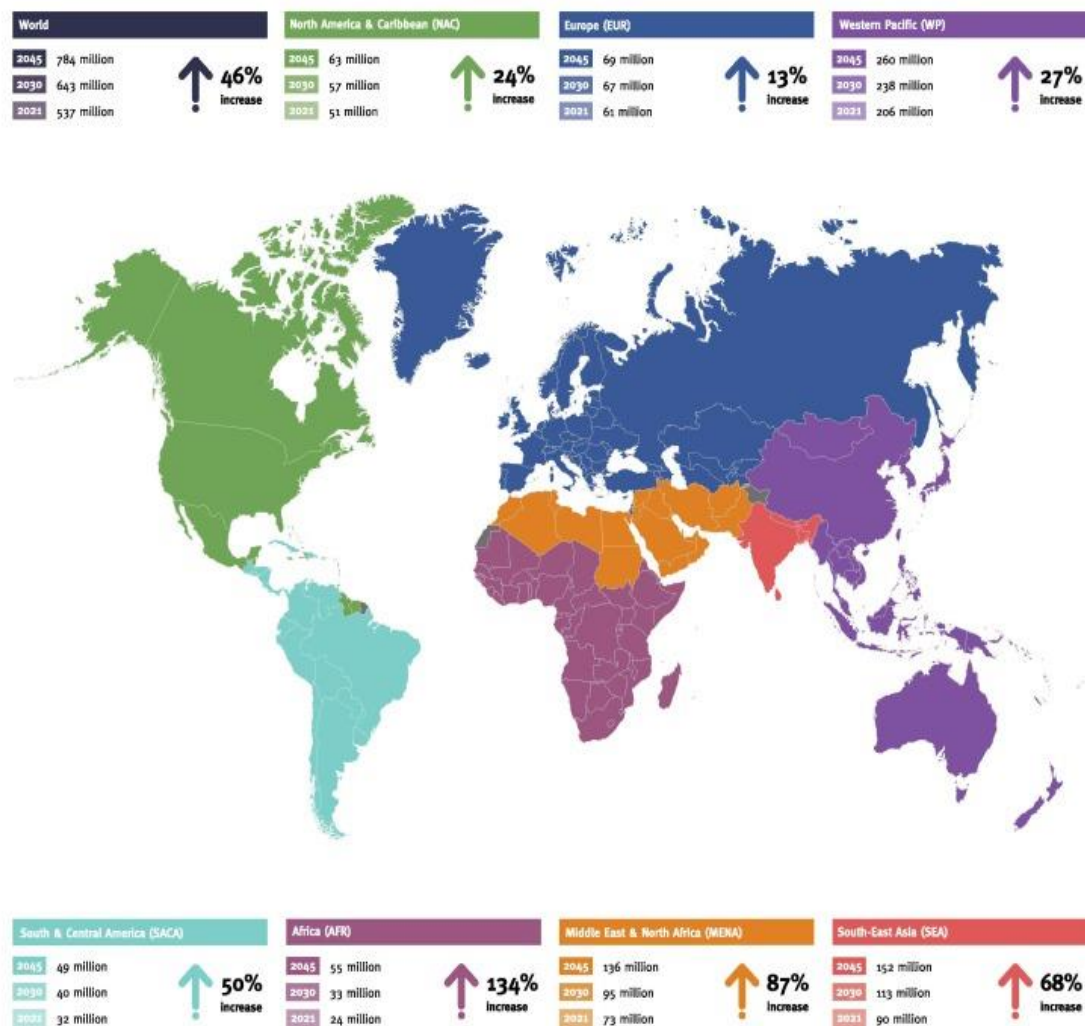
Figure 2.1: Global prevalence of diabetes mellitus.

Adapted from IDF diabetes atlas 10th edition (Sun *et al.*, 2022).

It is predicted that Africa has the greatest projection increase in both the burden of diabetes and diabetic related complications but in spite of this, it contributes the lowest in the global annual healthcare expenses towards diabetes care. Global diabetes-related health expenditures were estimated at 966 billion USD in 2021, and are projected to reach 1,054 billion USD by 2045 (Sun *et al.*, 2022). People with diabetes are bound to experience more than one chronic illness and a remarkable portion of the costs related with these complications are ascribed to the underlying diabetes. The majority of existing studies in Africa estimated only the medical direct costs. Nevertheless, the estimations of costs of diabetes in many countries in Africa may be underestimated owing to lack of data on the corresponding contribution of cost of diabetes complications. Approximately USD 13 billion was spent in Africa on healthcare for people with diabetes in 2021 translating to 1% of the total global expenditure on diabetes (Mapa-Tassou *et al.*, 2019; Sun *et al.*, 2022).

The economic burden of diabetes mellitus in Kenya depends on the cost of illness approach. The approach pinpoints and measures all the costs of diabetes mellitus, comprising direct and indirect costs. The 552,400 diabetes adult cases reported in 2019 lead to a total economic cost of $ 372,184,585, similar to $ 674 per diabetes mellitus patient. The total direct costs resulted in highest proportion of the gross costs at 61% (USD 227,980,126), while in contrast indirect costs accounted for 39% of the overall economic costs of $ 144,204,459 (Adamjee *et al.*, 2022) . Costs of purchasing medicines presented the highest costs over the entire economic costs at about 29%, followed by the income lost while looking for medical care at 19.7%. The other costs include productivity losses (19%), diagnostic tests (13%), travel (12%) and the rest of cost categories (5%) of

the general costs. Therefore, efforts need to be made to bring down the costs of these quality medicines to strengthen and sustain the medical care. The high indirect costs reported, income lost by patients while seeking medical care (indirect costs) is at 19% (Oyando *et al.*, 2020). Access to inexpensive health care services such as diabetes mellitus education, regular blood glucose testing initiatives, and expanding local manufacturing of medicines can lower the economic burden of diabetes mellitus and boost the health effects of the population and their input to the society (Adamjee *et al.*, 2022). Estimated financing gap generated by the difference between the available resources and the cost of implementing the NCD diabetes Strategy reveals that the Kenya's health sector requires more funds to reduce the funding gap. To bridge this gap, resources are mobilized from other sources like the donors and or the private sector including the households (Sujha Subramanian *et al.*, 2018).

2.4 Etiology of diabetes mellitus

Diabetes is associated with persistent high fasting glucose concentrations in the blood exceeding a certain level (>7mmol/L) that occurs as a result of impaired insulin secretion and/or resistance to peripheral actions of insulin (Czech, 2017). Environmental and genetic factors are responsible for the evolution of diabetes mellitus. Type I diabetes is not a genetically predestined disease however an increased susceptibility can be inherited. Amalgamation of genetic susceptibility and environmental factors such as viral infection, toxins, or some dietary factors have been implicated as triggers for autoimmunity (Dong *et al.*, 2019). In T2DM, the response to insulin is diminished, and in this state, insulin is ineffective and is at first countered by an increase in insulin production to maintain

glucose homeostasis, but over time, insulin production decreases thus resulting in T2DM (Goyal *et al.*, 2018).

Insulin plays a central role in the regulation of glucose homeostasis and acts in a coordinated fashion on cellular events that include the regulation of ion and amino acid uptake, protein synthesis and degradation, gene transcription and mRNA turnover, and cellular growth and differentiation (Lemmer *et al.*, 2021). Reduced sensitivity of peripheral tissues to insulin occurs due to reduction in number of insulin receptors, 'down regulation' of insulin receptors. An impairment of insulin action is involved in many diseases including T2DM (Yaribeygi *et al.*, 2019).

T2DM being a complex and heterogeneous disease can be distinguished by a progressive decline in insulin action or defect in insulin signal transduction; in this case the tissues can't utilize glucose normally, that is associated with serum triglycerides enhancement, serum HDL reduction and sometimes LDL enhancement (Décio L Eizirik *et al.*, 2020). Abnormalities in the regulation of peroxides and transition metal metabolism are postulated to result in establishment of the disease as well as its long term complications. Diabetes mellitus is associated with oxidative reactions, particularly those which are catalyzed by decompartmentalized transition metals, but their causative significance in diabetic tissue damage remains to be established (Korac *et al.*, 2021). It has been suggested that T2DM originates from an interaction between genetic and environmental factors such as physical activity and diet. Together these factors influence glucose metabolism and the development of DM (Beulens *et al.*, 2022).

The etiology of Type 1 diabetes involves both genetic and environmental factors. The genes implicated are 'susceptibility genes', which modify risk (Robertson *et al.*, 2018).

Individual susceptibility genes may not be required and are not sufficient for disease development. The strongest genetic risk component is encoded within the major histocompatibility complex (MHC) and is designated IDDM1. The HLA-DQ genes contribute to the risk, but so many other MHC-encoded genes(Robertson *et al.*, 2018). On the other hand, glucose tolerance deteriorates in human pregnancy however GDM is not due to diminished secretion of insulin or disproportional secretion of proinsulin or glucagon, nor is an increased insulin degradation involved. Poor nutrition, such as energy-dense western diets may cause overweight and obesity, disrupting insulin signaling pathways and insulin sensitivity. Saturated fats can disrupt insulin signaling; induce inflammation and endothelial dysfunction, resulting in GDM (Yahaya *et al.*, 2020).

2.5 Risk factors associated with diabetes mellitus

The main risk factor of diabetes mellitus is obesity. Studies have proved that being obese increases the risk of developing T2DM by 90-fold and that majority of type 2 diabetic patients are overweight or obese (Reed *et al.*, 2021). The genesis of obesity is too much energy intake and ensuing storage as well as poor energy expenditure leading to weight gain. Obesity has turned into an enormous healthcare burden over time, especially in LMICs. Nonetheless, excessive dietary intake may have a genetic etiology, as in leptin deficiency seen in some obese subjects. Obese individuals have a BMI of ≥ 30 kg/m^2, and individuals with a BMI of ≥ 25 and <30 are categorized as overweight. There is always a direct proportional relationship between BMI and both fasting and postprandial insulin levels. Such a relationship also exists between BMI and the extent of insulin resistance. The hyperinsulinemia linked with rising BMI is required to reduce insulin

resistance and regulation of blood glucose homeostasis (Reed *et al.*, 2021). Type 2 diabetes mellitus is hypothesized to be an outcome of the interaction of environmental, biological, and behavioral risk factors. Body mass index (BMI), lipids, hypertension**,** smoking, physical inactivity, low education, dietary patterns, family history, and recently also specific genes are the most frequently documented risk factors for type 2 diabetes mellitus (Dendup *et al.*, 2018).

2.6 Pathogenesis of diabetes mellitus

The pathogenesis of type 2 diabetes is intricate involving progressive development of insulin resistance in liver and peripheral tissues accompanied by a defective insulin secretion from pancreatic beta cells resulting to decrease in the metabolic responses to insulin leading to overt hyperglycemia (ADA, 2010; Skyler *et al.*, 2017). Under normal physiological conditions, plasma glucose concentrations are maintained within a narrow range, despite wide fluctuations in supply and demand, through a tightly regulated and dynamic interaction between tissue sensitivity to insulin (especially hepatic) and pancreatic islets insulin secretion. In type 2 diabetes mellitus, these mechanisms malfunction, leading to pathophysiological defects which include impaired insulin secretion through a dysfunction of the pancreatic beta-cell, and impaired insulin action through insulin resistance. Insulin resistance is the incapability of insulin to produce its usual biological effects at physiological concentrations and it is a primary feature of T2DM (Gonzalez-Rellan *et al.*, 2022; Petersen *et al.*, 2018). Pancreatic β-cell dysfunction results from inadequate glucose sensing to stimulate insulin secretion therefore elevated glucose concentrations remain high. Persistently elevated glucose concentrations above

the physiological range result in the manifestation of hyperglycemia that characterizes type 2 diabetes mellitus (Rorsman *et al.*, 2018).

The decreased insulin-stimulated glucose uptake is due to impaired insulin signaling and multiple post receptor intracellular defects including impaired glucose transport and glucose phosphorylation, and reduced glucose oxidation and glycogen synthesis (Fan *et al.*, 2023). Although the exact mechanism that leads to the development of insulin resistance in skeletal muscle is not yet fully understood, an increased intramyocellar fat content and fatty acid metabolites have been shown to play a pivotal role in the development of insulin resistance in skeletal muscle (Thaane *et al.*, 2019).

Type 1 diabetes is the result of an autoimmune reaction to proteins of the islets cells of the pancreas. A puzzling aspect of T1DM is that the immune system destroys pancreatic β-cells but not neighbouring α-cells, even though both β-cells and α-cells are dysfunctional. Anomaly, however, progresses to death only for β-cells (Decio L Eizirik *et al.*, 2023). There is a strong association between IDDM and other endocrine autoimmunity (for example, Addison disease) and an increased incidence of autoimmune diseases are seen in family members of IDDM patients. The three types of autoantibodies known are:

i. Islet cell cytoplasmic antibodies (ICCA): The primary antibodies found in 90% of type 1 diabetics are against islet cell cytoplasmic proteins. The presence of ICCA is a highly accurate predictor of future development of IDDM.

ii. Islet cell surface antibodies (ICSA): Autoantibodies directed against islets cell surface antigens (ICSA) have also been described in as many as 80% of type 1

diabetics. Some patients with type 2 diabetes mellitus have been identified, which are ICSA positive.

iii. Specific antigenic targets of islet cells: Antibodies to glutamic acid decarboxylase (Roep *et al.*, 2021; Yau *et al.*, 2021) have been identified in over 80% of patients newly diagnosed with IDDM. Anti-GAD antibodies decline over time in type 1 diabetics. The presence of anti-GAD antibodies is a strong predictor of the future development of IDDM in high risk populations. Anti-insulin antibodies (IAAs) have been identified in IDDM patients and in relatives at risk to developing IDDM. These IAAs are detectable even before the onset of insulin therapy in type 1 diabetics. IAA is detectable in around 40% of young children with IDDM (Ifeanyi, 2018).

The reason why pregnancy is capable of inducing temporary diabetic state is partly unknown. However, among the possible explanations are reduced insulin secretion, increased insulin degradation, elevated secretion of hormones with an anti-insulin effect (particularly glucagon, human placental lactogen [HPL], estrogens, progesterone, and cortisol), reduced tissue sensitivity to insulin, or a combination of two or more of the later mechanisms (Moyce Gruber *et al.*, 2023; Rana *et al.*, 2022) .

2.6.1 Role of insulin signaling in diabetes mellitus

The insulin receptor (IR) is a 320 kDa membrane receptor tyrosine kinase mediating the pleiotropic actions of insulin, leading to phosphorylation of several intracellular substrates including serine/threonine-protein kinase (AKT1), and IR autophosphorylation (Mendoza *et al.*, 2023). As shown in Fig. 2.3, insulin signaling happens when insulin

binds to the integral membrane α subunit of insulin receptor then receptor is activated, resulting in a conformational change known as dimerization (the coming together of two insulin receptors). The receptor then phosphorylates amino acids (tyrosines) on the tail of the other insulin receptor in the pair (activation of the intrinsic kinase activity of the β subunits). Signal transduction proteins interact with phosphate group and this interaction of the phosphate groups with all of the different signaling proteins occurs simultaneously. Signaling proteins and pathways causes transient and long-term changes in response to the increased glucose in the blood stream. One key short-term change is the fusion of vesicles containing glucose transporter (GLUT4) to the cell membrane and once these transporters are part of the cell surface, glucose is transported into the cell (Barbosa-da-Silva *et al.*, 2014; Bershatsky *et al.*, 2023; Copps *et al.*, 2012).

 Long-term cellular changes are caused by changes in gene transcription that result in specific proteins being made or not made. These pathways utilize many different signaling patterns, such as the direct activation of a transcription factor (T.F.), the release of second messenger, and the activation of a kinase cascade. All of these signaling pathways can result in the activation of transcription factors and their movement to the nucleus to activate transcription (Song *et al.*, 2019)

Some of the documented changes in insulin signaling as a result of diabetes mellitus (Figure 2.2) are:

- Decreased insulin receptor kinase activity upon binding of insulin to the receptor.

- Decreased binding of signaling effectors to the insulin receptor due to less phosphorylation sites on the insulin receptor and/or feedback inhibition on the signaling molecule, preventing its binding to the insulin receptor.

- Decreased signaling effector binding leads to defective downstream activation of the kinase cascade and second messenger signaling pathways.

- Decreased GLUT4 (glucose transporter) fusion to the cell membrane and less glucose transported into the cell (De Felice *et al.*, 2022; Rok Herman *et al.*, 2022).
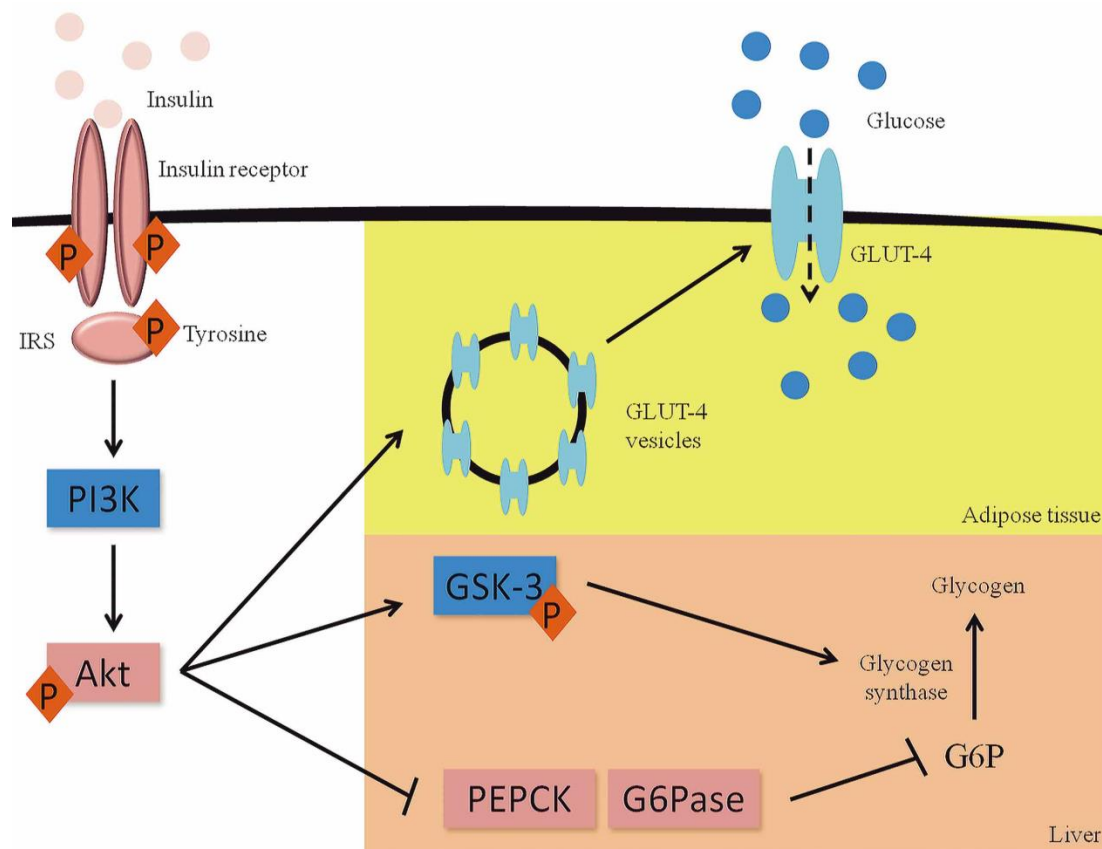
Figure 2. 2: Insulin signalling pathway.

Adapted from (Barbosa-da-Silva *et al.*, 2014).

Insulin signalling starts when insulin binds to its receptor (insulin receptor). Consequently, IRS 1/2 is phosphorylated in tyrosine, what leads to the downstream activation of PI3K and Akt. In white adipose tissue and skeletal muscle, Akt stimulates GLUT4 translocation to cell membranes thus allowing glucose to enter the cell. In liver, insulin stimulates glycogen production through phosphorylation of GSK-3 and consequently dephosphorylation of glycogen synthetase. Moreover, insulin inhibits gluconeogenesis by reducing enzymes of gluconeogenesis. Full arrows represent stimulation, dotted arrows represent transport and interrupted lines represent inhibition.

G6P= glucose-6- phosphate; G6Pase= glucose-6-phosphatase; GLUT4= glucose transporter type 4; GSK-3= glycogen synthase kinase 3; IRS= insulin receptor substrate.

2.6.2 Fructose metabolism and its role in T2DM

Fructose is a six-carbon monosaccharide sugar that is found in fruits and honey with a similar chemical formula with glucose. This could be the rationale why fructose too, also passes by the same metabolic pathway as glucose. Fructose and sucrose are commonly used as sweeteners in the medical and food industry. Fructose is predominantly used commercially because of its sweetening power of 1.5 to 1.7 times that of sucrose (Ang *et al.*, 2018).

Fructose gets into the cells by facilitated diffusion on the transmembrane GLUT5 transporter. Expression of GLUT5 in the intestine is upregulated by fructose and not sucrose. As it enters the enterocyte, fructose diffuses into the blood vessels through a transport orchestrated by GLUT2 at the basolateral membrane pole of the enterocyte (Merino Antolín *et al.*, 2020). GLUT8 has also been proven to regulate enterocyte fructose transport. DeBosch et al., 2012 tested the hypothesis that GLUT8 regulates intestinal hexose uptake and metabolic homeostasis *in vivo* mice and demonstrated that GLUT8 do not contribute to the fructose uptake (DeBosch *et al.*, 2012). In the liver, fructose is catabolized to pyruvate or under fasting conditions to glucose and then metabolized to intermediates of glycolysis. Either hexokinase or fructokinase catalyzes the phosphorylation of fructose and it is through this reaction that mediates fructose entry to the intermediary metabolism(Mark A Herman *et al.*, 2021). Then fructose 1-phosphate is formed and then cleaved by aldolase B to form intermediates of glycolysis; dihydroxyacetone phosphate (DHAP). Additionally, DHAP may be reduced to glycerol-

3-phosphate to be a mainstay for de novo lipogenesis (DNL), allowing the synthesis of triglycerides (TG) and lipoproteins. Another intermediate is glyceraldehyde, which is phosphorylated by ATP to form glyceraldehyde 3-phosphate. If not, the fructose can be converted to glucose by gluconeogenesis(Hannou *et al.*, 2018). Fructose metabolism parallels (bypasses the phosphofructokinase regulatory pathway) that of glycolysis. When it becomes pyruvate, it enters the Krebs cycle and fatty acid synthesis thus increases hepatic triglyceride production. This is the reason why excess fructose can cause obesity, thereby affecting T2DM (Dewdney *et al.*, 2020). The basic metabolic differences in glucose and fructose metabolism have been well summarized in Figure 2.3 (Federico *et al.*, 2021a; Hannou *et al.*, 2018).
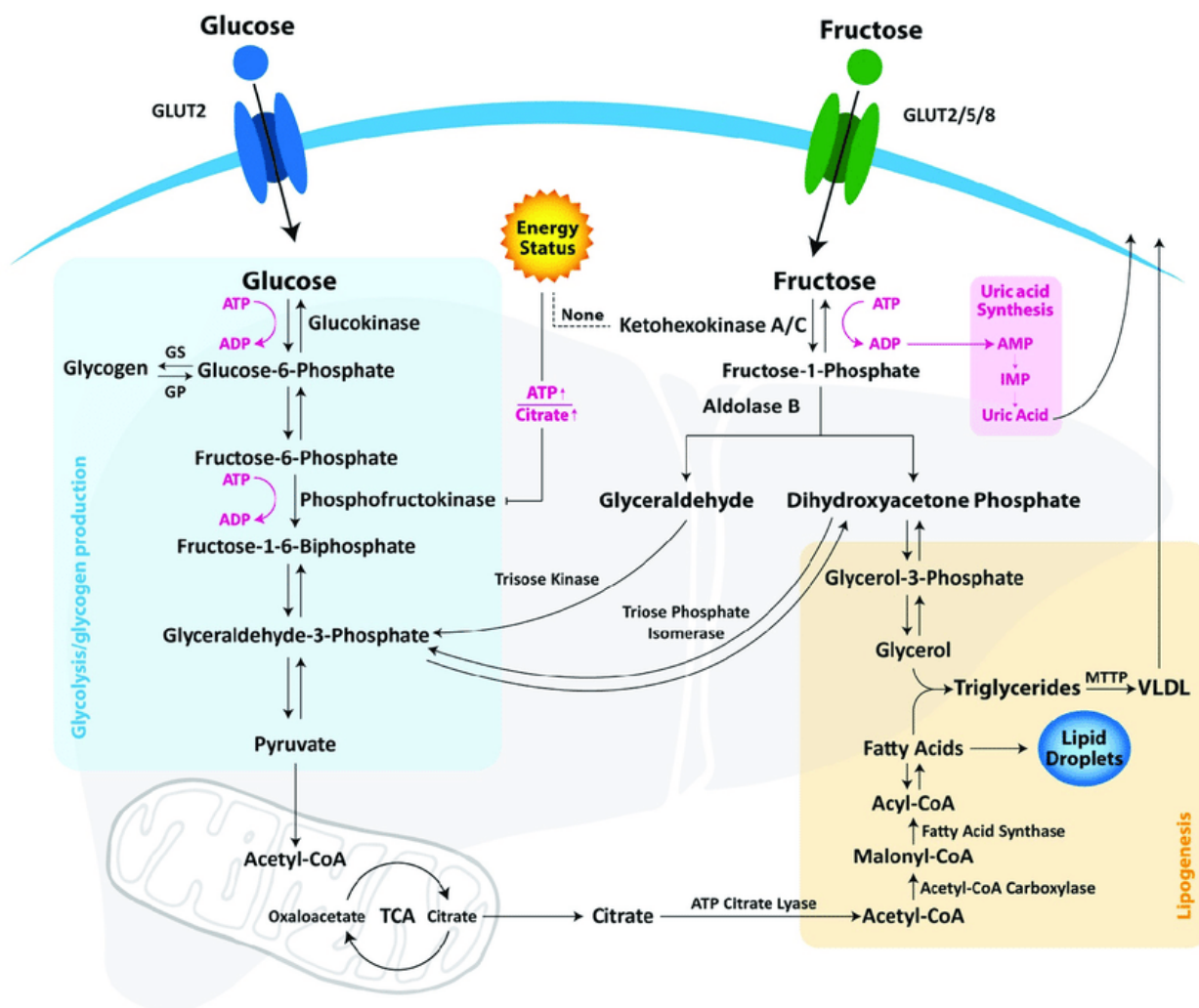
Figure 2.3: Glucose and fructose metabolism.

Adapted from (Federico *et al.*, 2021b)

Glucose is transported into the hepatocyte via GLUT 2 and is successively metabolized though glycolysis to produce ATP and pyruvate or is stored as glycogen. Fructose can be transported via GLUTs2, 5, and 8 and is phosphorylated by ketohexokinase, A or C, into fructose-1-phosphate, that is cleaved in glyceraldehyde and dihydroxyacetone phosphate. While glucose metabolism is regulated by phosphofructokinase, which is inhibited by

ATP and citrate when energy status is high, the ketohexokinase activity is unrestricted. Glyceraldehyde and dihydroxyacetone phosphate are phosphorylated, respectively, by triose kinase or triose phosphate isomerase in glyceraldehyde-3-phosphate to produce pyruvate for fuel triglyceride synthesis or fructose-1,6-biphosphate for gluconeogenesis. Dihydroxyacetone phosphate may be converted in glycerol-3-phosphate to production of triglycerides and VLDL lipoproteins. ADP, adenosine diphosphate; ATP, adenosine triphosphate, AMP, adenosine monophosphate; IMP, inosine monophosphate; Glut, glucose transporter; Gp, glycogen phosphorylase; Gs, glycogen synthase; MTTP, microsomal triglyceride transfer protein; TCA,citric acid cycle; VLDL, very low-density lipoprotein.

Hypertriglyceridemia in fructose-treated animals has been proposed, caused by either VLDL-TG enhancement due to an increased hepatic secretion of them or a decreased elimination of TG-rich lipoproteins from the circulation (Anvari *et al.*, 2014). Serum triglycerides and cholesterol enhancement are the major factors of IR induction. Entrance ability of glucose to the cells and its oxidation was inhibited by fatty acids through the glucose transporters activity impairment and change of the enzymatic activity of glycogen synthesis. Thus, leading to prolonged hyperglycemia and impaired insulin action (Chadt *et al.*, 2020).

2.7 Symptoms and diagnosis of diabetes mellitus

The clinical diagnosis of diabetes is usually occasioned by symptoms including: increased thirst, hunger and urine volume (frequent urination), recurrent infections, certain vision changes, unexplained weight loss and in severe cases, drowsiness and coma (Balaji *et al.*, 2019).

The diagnostic criteria of DM is based on (i) measured variable venous plasma glucose: Random plasma glucose value of $\geq$ 200mg/dL ($\geq$ 11.1mmol/L), Fasting plasma glucose of $\geq$ 126mg/dl (7.0mmol/L) (fasting time 8–12 h), or OGTT 2-h value in venous plasma $\geq$ 200mg/dL ($\geq$ 11.1mmol/L) and (ii) Measured variable HbA1c: HbA1c $\geq$ 6.5% ($\geq$ 48mmol/mol Hb).

Abnormally elevated fasting glucose levels are characterized by IFG (impaired fasting glucose) for the fasting glucose range of 100– 125mg/dL (5.6mmol- 6.9mmol/L) in venous plasma. Disturbed glucose tolerance is represented by an IGT (impaired glucose tolerance) that corresponds to a 2-h plasma glucose value in OGTT in the range of 140-199mg/dL (7.8-11.0mmol/L) with fasting glucose values of $<$ 126mg/dL ($<$ 7.0mmol/L). Most people who presents with glucose tolerance disorder have IFG and IGT (Petersmann *et al.*, 2019; Severeyn *et al.*, 2019).

Single blood glucose estimation in excess of the diagnostic values indicated above, establishes the DM diagnosis in such cases. An oral glucose tolerance test (OGTT) to establish diagnostic status need only be considered if casual blood glucose values lie in the uncertain range and fasting blood glucose levels are below those which establish the diagnosis of DM. If an OGTT is performed, it is sufficient to measure the blood glucose values while fasting and at 2 hours after a 75 g oral glucose load (ADA, 2010).

Glycated hemoglobin (HbA1c) is another test for diagnosing DM together with fasting blood sugar. HbA1c measurement is approved by the American Diabetes Association for the diagnosis and monitoring of DM in human patients. It is also useful diagnostic assay for the determination of individuals at high risk of developing diabetes mellitus. HbA_{1c} has major advantages over glucose testing as it lacks variability and has

convenience of testing. However, in many countries the test is not sufficiently standardized and also a costly test (Association, 2017). HbA1c represents the percentage of circulating hemoglobin that is glycated. Glycation is a non-enzymatic process and is a measure of glucose levels over time. As a biomarker, it reflects the average plasma glucose over the previous 8–12 weeks. It is currently used for both the diagnosis and management of diabetes and is recommended as a gold standard in the assessment of diabetes-related outcomes. It is widely used to judge the adequacy of diabetes treatment. Nevertheless, for a given HbA1c, there is a wide range of mean glucose concentration values, and for any given mean glucose value there is a wide range of HbA1c values (Chehregosha *et al.*, 2019).

2.8. Complications of diabetes mellitus

Diabetes mellitus is associated with complications such as hypertension, endothelial damage, cardiac hypertrophy, inflammation, atherosclerosis, ventricular contractile dysfunction, fibrosis, retinopathy, and nephropathy (Verhulst *et al.*, 2019). Vascular complications of type 2 diabetes account for the majority of the social and economic burden among patients and society more broadly (Dal Canto *et al.*, 2019). Approximately one half of patients with type 2 diabetes mellitus die prematurely of a cardiovascular cause and approximately 10% die of renal failure. Cardiovascular disease (CVD) is the most significant cause of death in the diabetic population. Among persons with diabetes, part of the increased likelihood of cardiovascular disease seems to be a consequence of the increased frequency of such risk factors as hypertension, high lipids in the blood, and physical inactivity (Dal Canto *et al.*, 2019). Diabetic retinopathy is a leading cause of blindness among adults aged 20–74 years. Diabetics are six times more prone to cataracts

and 1.4 times more susceptible to open-angle glaucoma when compared to the general population (Bariuad-Garcia *et al.*, 2022). Diabetic neuropathy may present without symptoms or pain, sensory loss, weakness, or autonomic dysfunction. The condition may lead to a significant morbidity and may advance to other major complications, such as lower extremity amputation, which is the main debilitating complication (Jensen *et al.*, 2021). Poor blood glucose control, diastolic hypertension, infection, dyslipidemia, and poor self-care are risk factors linked to diabetic foot ulcers but are modifiable and manageable (Association, 2023). Diabetic ketoacidosis (DKA) develops when absolute insulin deficiency and an absolute increase in contra-insulin hormones, increasing hepatic glucose output, reduced peripheral glucose utilization, and stimulating release of fatty acids from adipocytes and ketogenesis by the liver are present (Dhatariya *et al.*, 2020).

The exact mechanism by which the diabetic metabolic state cause microvascular and macrovascular complications is only partially understood but most likely involves both uncontrolled BP and uncontrolled glucose, increasing the risk of microvascular complications such as retinopathy and nephropathy (Prajjwal *et al.*, 2023). Mechanisms may involve errors in aldose reductase and other metabolic pathways, damage to tissues from accumulation of glycated end products, and other mechanisms (Ogunsanmi *et al.*, 2022). In regard to macrovascular complications, high BP and glucose levels raise risk, but so do lipid abnormalities and tobacco use. One unifying theory suggests the existence of a metabolic syndrome that includes diabetes mellitus, hypertension, dyslipidemias, and obesity, and predisposes to coronary heart disease, stroke, and peripheral artery disease (Swarup *et al.*, 2022).

2.9 Treatment and management of diabetes mellitus

The primary goal of treatment is to bring down the elevated blood glucose to a normal range, both to improve symptoms of diabetes and to prevent or delay diabetic complications (Balaji *et al.*, 2019). Treatment should also focus on the correction of any associated cardiovascular disease risk factors such as smoking, hyperlipidemias, and obesity as well as monitoring of blood pressure and treatment of hypertension (Butt, 2022).

There are three main types of diabetes therapeutic modalities: (1) insulin only; (2) combination of insulin and one or more oral antidiabetic drugs (OADs) and/or glucagon-like peptide-1 receptor agonists (GLP-1RAs); and (3) treatment with one or more OADs and/or GLP-1RAs without insulin (Doucet *et al.*, 2023). Insulin replacement therapy is the mainstay for patients with type 1 diabetes mellitus and treatment of all type 1 diabetic patients requires administration of exogenous insulin (Tatovic *et al.*, 2023) while diet and lifestyle modifications are fundamental in the treatment and management of type 2 diabetes mellitus in its early stages. Insulin is also crucial in type 2 diabetes mellitus patients who do not achieve adequate blood glucose levels by managing diet, reducing weight, exercising and using oral hypoglycemic medications (Mahgoub *et al.*, 2023).

2.9.1 Oral antidiabetic drugs and their limitations in the treatment of diabetes mellitus

The modern existing oral hypoglycaemic drugs are classified into different groups based on their mechanisms of action. These hypoglycemic drugs include but is not restricted to sulfonylureas, meglitinides, biguanides, thiazolidinediones, alpha-glucosidase inhibitors and dipeptidyl peptidase 4 (DPP-IV) inhibitors (Mahgoub *et al.*, 2023).

As shown in Figure 2.4 the sulfonylureas stimulate insulin release from the pancreatic β-cells. They bind to the SUR-1 (sulfonylurea receptor-1), expressed on the pancreatic β-cell membranes, as a result inhibits the efflux of potassium ions through the gated-channels that cause membrane depolarization (Da Costa Mousinho *et al.*, 2013). This depolarization initiates the opening of voltage-gated calcium channels, resulting to increased influx of calcium, and insulin release is stimulated by the elevated levels of intracellular calcium (Velasco *et al.*, 2016). These antidiabetic drugs however do not decrease the long-term complication of diabetes and in addition ameliorates appetite thus resulting in diabesity (Lazzaroni *et al.*, 2021). Examples of SUs are tolbutamide, tolazamide, acetohexamide chlorpropamide, glyburide, glipizide, glibenclamide and glimepiride.
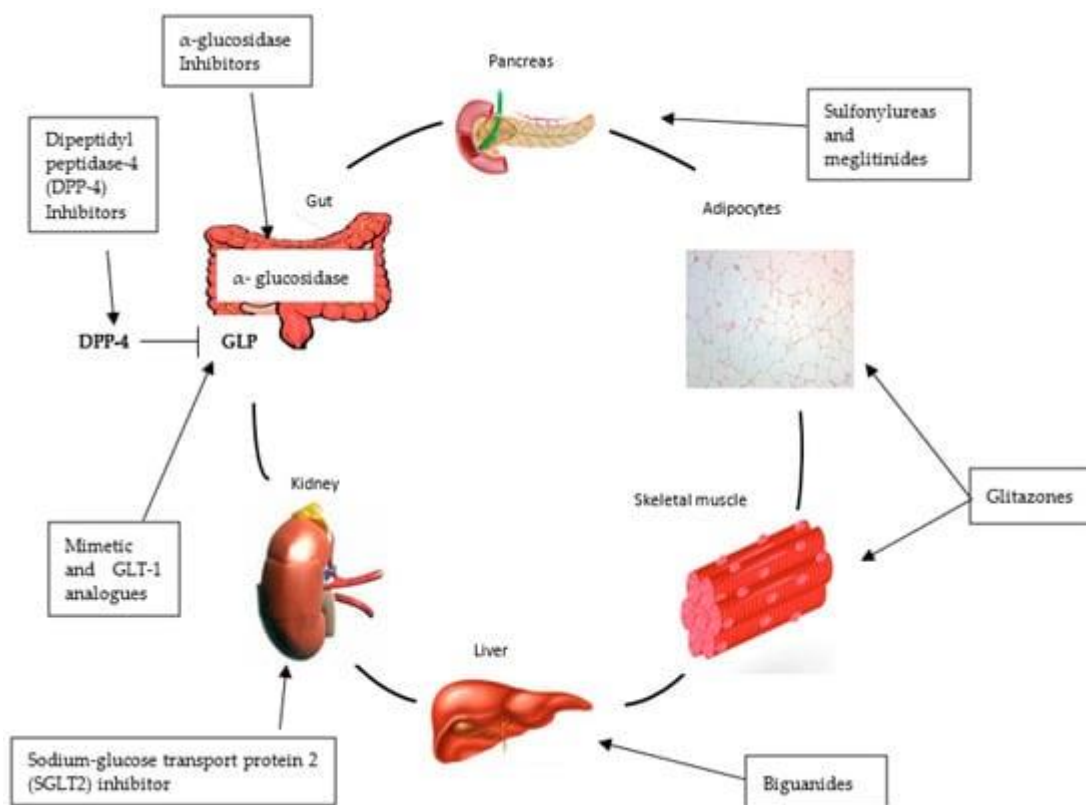
Figure 2.4: The target tissues of current antidiabetic drugs.

Adapted from (Sagbo *et al.*, 2022).

Meglitinides are OADs that lowers blood sugar levels by increasing insulin release from the beta-cell pancreatic islets (Abdelhamid *et al.*, 2022). This is realized by modulating beta cells to secrete insulin by controlling the efflux of potassium through potassium channels. Meglitinides do not have a direct effect on insulin emeicoytosis as it is in sulfonylureas (Timmons and *et al.*, 2022). Weight gain and hypoglycaemia are some of the reported side effects associated with meglitinides. The classical examples of the antidiabetic drugs in this category are repaglinide and nateglinide (Scheen, 2021).

Biguanides are another class of OADs that works by improving insulin sensitivity in peripheral tissues through the modification of post-receptor signalling in the insulin

signalling pathway. The effects of this class of OADs on hepatic tissue result in reduced hepatic glucose output through a decrease in gluconeogenesis and glycogenolysis (Tupas *et al.*, 2020). Metformin is the best example of this class, though it has been reported to have known adverse side effects such as heart failure, hepatic impairment, gastrointestinal disturbances and renal impairment (Sanchez-Rangel *et al.*, 2017).

The thiazolidinediones achieves their antidiabetic activity by binding to the PPARγ (peroxisome proliferator-activated receptor gamma) mainly expressed in adipocytes. Binding to PPARγ enhances interaction with the retinoid X receptor, which hetero-dimerizes and activates transcriptional expression of genes that play a crucial role in lipid and carbohydrate metabolism (Cox, 2022). They improve muscle and fat sensitivity to insulin and, to a lesser extent, reduce hepatic glucose production. Pathophysiology of fluid-retention leads to oedema, heart failure, liver toxicity and anaemia and weight increase is the major limitations linked to this class of drugs. Examples of drugs in this class include troglitazone, rosiglitazone and pioglitazone (DeFronzo *et al.*, 2019).

The α-glucosidase inhibitor or starch blockers inhibits the action of intestinal enzymes responsible for breakdown of carbohydrates in the small intestine, thus slowing down the absorption of ingested carbohydrates and decreasing post-prandial hyperglycaemia in diabetic patients (Bhatnagar *et al.*, 2022). However, one of the major setbacks of these oral antidiabetic drugs is their side effects which include nausea, flatulence, diarrhoea, bloating and abdominal pains. Acarbose and miglitol are examples of this type of oral antidiabetic drugs (Mukhtar *et al.*, 2020).

The dipeptidyl peptidase 4 (DPP-IV) inhibitors are a group OADs that attenuate incretin degradation, thus increasing the half-life of incretin and enhancing the stimulation of

pancreatic insulin secretion and beta cell growth (Dahiya *et al.*, 2020). Incretin hormones (glucose-dependent insulinotropics peptide (GIP) and glucagon-like peptide-GLP-1) contribute importantly to glucose-dependent insulin secretion by increasing beta cell mass and limiting glucagon secretion. Adverse side effects of DPP-IV inhibitors include headache, runny nose, diarrhea, nausea, stomach pain, pancreatitis and sore throat. The best examples of this class are alogliptin, linagliptin, sitagliptin and saxagliptin (Andersen *et al.*, 2018).

2.9.2 Traditional herbal remedies in diabetes mellitus treatment and management

There is a long history of traditional plants used for the control of diabetes mellitus. Herbal drugs are proved to be a better option over synthetic drugs (Oral antidiabetic drugs) because they are usually less costly with fewer side and adverse effects. This has accelerated the use of medicinal plants that are considered to have multiple therapeutic targets and are easily accessible (Sagbo *et al.*, 2022). These drugs are also used when chemical drugs are ineffective in treatment of disease. Currently the medicinal plants and herbs are being used in extract forms for their anti-diabetic activity. Various clinical studies have confirmed that medicinal plant extracts shows anti-diabetic activity and restores the action of pancreatic β- cells (Verma *et al.*, 2018).

The field of herbal medicines research has been gaining significant importance in the last few decades and the demand to use natural products in the treatment of diabetes is increasing due to perceptions of safety and efficacy. Currently, over 400 traditional plant treatments for DM have been reported, although only a small number of these have received scientific and medical evaluation to assess their efficacy (Saggar *et al.*, 2022). Metformin is an effective oral glucose-lowering agent which was developed based on the

use of *Galega officinalis* to treat DM. *Galega officinalis* is rich in guanidine, the hypoglycemic component (Bailey, 2017) .

The hypoglycemic effect of some herbal extracts has been confirmed in human and animal models of type 2 diabetes mellitus (Hasan *et al.*, 2018). The World Health Organization Expert Committee on DM recommended that traditional medicinal herbs be further investigated (Kifle *et al.*, 2020). The major obstacle in the integration of herbal medicine into modern medical practices is the lack of scientific and clinical data proving their efficacy, mechanisms of action and safety (Parveen *et al.*, 2020).

Tarchonanthus camphoratus belongs to the family of *Asteraceae* and is found in a wide range of environments like Africa. Its common name is wild sage or camphor bush, (Nasr *et al.*, 2020) (Lelechwet is Kalenjin local name in Kenya). It is a small tree that grows to a height of 6 m with moderate to strong odour of camphor. It is widely spread in all the provinces of South Africa, Lesotho, Swaziland, and Namibia (Stehn, 2020). In Kenya, it is found in almost all the provinces. The leaves of the plant are alternate, borne on white-feted twigs, variable, 2 x 0.5 - 12 x 5 cm, obovate to lanceolate, upper surface smooth and finely reticulate with minute golden glands over the veinlets, lower surface white-felted margin entire to denticulate (Figure 2.5. Flowers are cream, borne on discoid heads in terminal or auxiliary panicles, female 1-3 flowered, male with numerous flowers; fruits are densely white woolly achene (Kenanda *et al.*, 2019) .

The leaves of this plant are used as decoctions or infusions, to relieve bronchitis, asthma, headache, inflammations, chilblains or abdominal pains. Aromatic medicinal plants produce a wide variety of volatile terpene hydrocarbons and essential oils. Essential oils

act as chemical defense against plant pathogens and thus benefits the plant too
(Nyakudya *et al.*, 2020).

 **Figure 2.5: A photograph of *Tarchonanthus camphoratus* taken at Longisa, Bomet
County.**

Tarchonanthus Camphoratus is traditionally known for various medicinal purposes. The
fresh leaves of this plant are used locally in Kenya for management diabetes. It is also
used to manage bronchospasm (Aro *et al.*, 2021). Additionally, bioactive high content of
parthenolide derived from *T. camphoratus* leaf extracts shows *in vitro* anticell
proliferative or cytotoxic potential against human hepatoma (i.e., HuH7 cells) cancer cell
lines (Siddiqui *et al.*, 2021). Other studies have reported that the aqueous and ethanol
extracts of *T. camphoratus* exhibits high glucose uptake in C2C12 muscle cells (Cock *et
al.*, 2021).

2.10 Prevention of diabetes mellitus

The primary prevention of diabetes mellitus should focus on controlling modifiable risk factors in a population such as a body weight, diet and lifestyle. Overweight is the most critical risk factor and should be targeted for prevention of type 2 diabetes especially among children and youths (Genovesi *et al.*, 2019). Maintaining a healthy body weight and avoiding weight gain during adulthood is the cornerstone of diabetes prevention since excess adiposity is the most important risk factor for diabetes. Weight loss improves pancreatic β-cell function and the sensitivity of liver and skeletal muscle to insulin, with larger relative weight losses leading to graded improvements in key adipose tissue disturbances (Magkos *et al.*, 2016).

Increasing physical activity and reducing sedentary lifestyle by developing an exercising habit has considerable potential to prevent or delay the onset of DM and improve insulin sensitivity (Matthias Li *et al.*, 2023). The American Diabetes Association (ADA) recommends that adults with diabetes participate in both aerobic activity and resistance training. They specify that this should entail at least 150 minutes of moderate-to-vigorous aerobic activity per week, spread over at least three days per week to minimize consecutive days without activity, and two to three sessions of resistance exercise per week on nonconsecutive days (Association, 2020). Regular exercise is associated with prevention and minimization of weight gain, reduction in blood pressure, improvement in insulin sensitivity and glucose control, and optimization of lipoprotein profile, all of which are independent risk factors for the development of T2D (Valenzuela *et al.*, 2021). Exercise is an established strategy for T2D prevention and the incidence of T2D is inversely proportional to participation in physical activity.

Modification of diet is another prevention mechanism of T2DM. High consumption of high glycemic index and glycemic load diet, red meat and processed meat, sugar and artificial sweetened beverages are some of the unhealthy diets that can accelerate the development of T2DM (Ruze *et al.*, 2023). However, some food groups and specific nutrients such as whole grain, low-fat dairy product, cheese, yogurt, olive oil, total fiber, dietary magnesium, and flavonoids are known to have beneficial effects and can produce synergistic health effects by decreasing calories through metabolic propensity toward abdominal adiposity particularly visceral fat, which in turn influences the insulin response (Toi *et al.*, 2020). Dietary interventions including low carbohydrate, low fat, and low consumption of sugar sweetened beverages have been shown to reduce body weight and visceral fat and thus reduces the risk diabetes mellitus (Alwash *et al.*, 2021)

2.11 Laboratory rodent models of diabetes mellitus

Laboratory animal models are been utilized in a wide range of biomedical research and translational studies in which spontaneous or induced pathological processes underlying human diseases can be investigated (Domínguez-Oliva *et al.*, 2023; Onaciu *et al.*, 2020). Animal models are a simple representation of a complex existing system and are essential in bridging the translational gap between preclinical and clinical research, as they provide rich means of testing hypotheses in a highly controlled environment. Furthermore, their phenomenon in one or more respects resembles the same phenomenon in humans(Vijay K Singh *et al.*, 2021).

Animal models have historically played an important role in the exploration and characterization of disease pathophysiology, target identification and evaluation of novel therapeutic agents and treatments in vivo. In T2DM, numerous animal models have been

developed for understanding the pathophysiology of diabetes and its complications (Kottaisamy *et al.*, 2021). These animal models tend to include models of insulin resistance and/or models of beta cell failure. On the other hand, several T2D animal models are obese, reflecting the human condition where obesity is closely linked to T2D development. Some of these models are induced using diet whereas others tend to have abnormalities in a single gene or multiple genes related to obesity, glucose intolerance, and/or insulin resistance leading to high blood glucose levels (Preguica *et al.*, 2020; Schoonejans *et al.*, 2021).

Many of the diabetes trials are performed in rodents while some studies are also done in larger animals. Rodents are preferred because they are cheaper and manageable. These experimental animals used in the study of diabetes mellitus can either be genetically diabetic animals, miscellaneous models or models based on the methods inducing experimental diabetes mellitus (Kottaisamy *et al.*, 2021). Diabetes can be induced in the experimental animals either by spontaneous methods or by using chemical agents or genetic manipulation. Both are of significant as they enable the analysis of particular mechanisms related to the disease and are important for understanding the pathogenesis and progression of the disease and extrapolating to humans (Onaciu *et al.*, 2020).

Wistar rats as experimental animal models of human disease offers various favorable circumstances and advantages over the mouse and different species (P Mukherjee *et al.*, 2022). The physiology in the rodent is simpler to follow and a lot of information is developed within a short period and this is the reason rats are extensively used as a suitable animal model for understanding the metabolic profile and pathology involved in different stages of T2DM (Foretz *et al.*, 2019).

The biological, genetic, and pathological implications of T2D are commonly investigated using animal models induced by a dietary intervention(Stott *et al.*, 2020). HFD consumed *ad libitum* results in rodents exceeding typical daily caloric intake. The exposure of the liver to such large quantities of fructose leads to rapid stimulation of lipogenesis and TG accumulation, which in turn contributes to reduced insulin sensitivity and hepatic insulin resistance/glucose intolerance (Eğritağ *et al.*, 2022). Studies have also shown that rats given the high fructose solutions had a higher serum TG levels and decreased ability to tolerate a glucose load (Busnatu *et al.*, 2022).

CHAPTER THREE

METHODOLOGY

3.1. Ethical considerations

The experimental protocols were approved by the Research Ethics Committee of University of Eastern Africa, Baraton (Reference; REC: UEAB/6/3/2017) as indicated in Appendix 2. The research was conducted in compliance with the ARRIVE guidelines 2019 and in accordance with the UK Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments and the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

3.2. Collection and identification of plant material

Leaves of *T. camphoratus* (TC) were harvested in December 2021 at Longisa area in Bomet County, Kenya (Latitude/Longitude (dec): -0.8667° S, 35.3833° E). The leaves were then packed and transported to University of Eldoret. The plant material sample was identified and authenticated by a qualified taxonomist, Mr. Denis Onyango at University of Eldoret and a voucher number MUH/TORC/013/1987 was assigned and the plant specimen kept in the herbarium of Department of Biological Sciences at University of Eldoret. The leaves were then dried at room temperature away from direct sunlight.

3.3. Preparation of plant crude leaf extract

The dried leaves were ground into fine powder using an electric miller (Disk Mill FFc-23, China). Dried powder (400 g) of TC plant were subjected to maceration in 4 litres of distilled water for 72 hours in a ratio of 1:10 (w/v). The organic extracts were then filtered using Whatmann No. 1 filter paper and the filtrate was then freeze-dried (LSL

Secfroid SR, Model 3021, Switzerland) for 24 hours as described by Amabeoku G. J. *et al.*, 2000. The paste obtained was put in a clean glass bottle and stored at -20° C until required for use. The dried extracts were freshly dissolved and made up to the appropriate volume with distilled water just before use on each day of the experiment.

3.4. Qualitative phytochemical analysis of leaf extract

Qualitative phytochemical screening of *T.camphoratus* leaf extracts was carried out as described below.

Test for saponins

This test was done as previously described by (Kokate, 1999)Briefly, 2 grams of the extract was dissolved in 6 mL of distilled water and shaken vigorously. Presence of saponins was determined by observing the formation of persistent 2 cm foam.

Test for tannins

The extract (0.5 grams) was put in a test tube and diluted with 3 mL distilled water. 3 drops of 5% ferric chloride was then added. Black or blue green coloration or precipitate indicated presence of tannins as described (Mojab *et al.*, 2003) .

Test for alkaloids

Two grams of the extract was dissolved in 5 mL of 1% hydrochloric acid then Wagner's reagent was added. Formation of a reddish brown precipitate indicated the presence of alkaloids as described by (Joshi *et al.*, 2013)

Test for flavanoids (Shibita's reaction test)

One gram (1 g) of the water extract was dissolved in methanol (50%, 2 mL) by heating, then metal magnesium and 5 - 6 drops of concentrated HCl were added. The solution when red was indicative of flavonols presence and orange for flavones as described by (Auwal *et al.*, 2014).

Test for terpenoids

One gram of crude extract was separately shaken with 2 mL of chloroform then followed by 2 mL of concentrated sulphuric acid along the side of the test tube. A reddish brown coloration interface indicated the presence of terpenoids which is the basis of this Salkowski test as described by (Ayoola *et al.*, 2008)

Test for steroids

One gram of the extract was dissolved in 3 mL of chloroform and equal amount of concentrated sulphuric acid was added by the side of the test tube. Reddish upper layer and yellowish sulphuric acid layer with green fluorescence indicated the presence of steroids as described by (Hossain *et al.*, 2013).

 Test for cardiac glycosides

This was done according to the Keller-Killiani test. In brief, 0.5 g of the extract was shaken in 3 mL distilled water then 2 mL of glacial acetic acid containing 3 drops of ferric chloride was added, followed by 1 mL of concentrated sulphuric acid along the side of the test tube. The formation of brown ring at the interface was used as a positive indicator for cardiac glycoside as described by (Iqbal *et al.*, 2015) .

Test for phenolic compounds

One gram of the extract was dissolved in 1mL of distilled water. To this solution 3 mL of 10% lead acetate solution was added. A dark green color indicated the presence of phenolic compounds as described by (Harborne, 1998)..

3.5. Experimental animals

Thirty-five (35), 6- to 8-week-old male Wistar rats (*Rattus norvegicus*) of similar weights (mean weight variation not exceeding ± 10 g) were obtained from the animal house of the Biology Department, Chiromo Campus, University of Nairobi, Kenya. The wistar rats were transported to University of Eldoret animal facility where they were kept in cages and allowed to acclimatize for 1 week before experimentation. During this acclimatization period, the rats were fed with commercial formulated rat feed (regular rodent chow) and water *ad libitum.* Also the wistar rats were exposed to environmental temperature (25 ± 2˚C), 40-60% room humidity and natural day and night circles of 12h/12h.

3.6 Study diet and induction of type 2 diabetes mellitus

After 2 weeks of acclimatization, all the wistar rats were labeled by tail tattooing using indelible ink, then their baseline fasting body weights were taken and their blood glucose measured using a glucometer (On-Call Plus, Acon Laboratories, San Diego USA) and was done by drawing whole blood from the tail vein following a 14 hrs overnight fast. The rats were then initially distributed into five groups (*n=35*) with similar baseline mean blood glucose values; One (1) of this groups was then randomly selected to represent the normal control group (*n=7*). The remaining (*n=28*) formed the experimental groups. The normal group (normal control) was fed with normal rodent chow (Rat chow pellets, Unga

Farmcare, East Africa Limited, Nakuru, Kenya) with free access to drinking water while the experimental group was fed a high fructose diet (HFD) consisting of standard rodent chow supplemented with 25% fructose (w/v) in drinking water for twelve weeks to induce type 2 diabetes mellitus. At the end of the 12 weeks feeding with HFD, fasting blood glucose and weights were measured.

3.7. Preparations of TC extract stock solution and dosage calculations

TC stock solutions of low dose (300 mgkg^{-1}bw^{-1}) and high dose (600 mg kg^{-1}bw^{-1}) were prepared each separately. Stock solution was prepared by dissolving 1 gram of TC extract in distilled water to obtain a stock solution of 60 mg/mL. At a dose of 300 mgkg^{-1}bw^{-1}, a rat weighing 100g was given 30 mg i.e. 0.5 mL stock solution while a rat weighing 150 g received 45 mg i.e. 0.75 mL stock solution. At a dose of 600 mgkg^{-1}bw^{-1} ; a rat weighing 100 g was given 60 mg i.e. 0.6 mL stock solution 60 mg/0.6mL while a rat weighing 150 g received 90 mg i.e. 0.75 mL stock solution according to OECD's guidelines 407 (Repeated Dose 28-Day Oral Toxicity Study in Rodents). For metformin tablets (Glucophage, Lipha Pharma Ltd., Middlesex city UK), 100 mg tablet was dissolved in 1 mL (100 mg/mL) of distilled water as described by Juei-Tang Cheng *et al.*, 2006 for oral administration (100µL) into rats (OECD.,1995 No.407-409) at 100 mg/kg bw^{-1} by gavage daily for the entire treatment period.

3.8 Sample size determination

The minimum number of animals to be used in the study was determined according to (Arifin *et al.*, 2017; Charan *et al.*, 2013).

The following formula was applied in sample size calculation:

$DF = N - k = kn - k = k(n - 1)$, 40-5 = (5×8) -8 = 5(8-1) = 35

Where DF is the degree of freedom of ANOVA, N = total number of subjects, k = number of groups, and n = number of subjects per group. The value of DF should lie between 10 and 20. If DF is less than 10 then adding more animals will increase the chance of getting more significant result, but if it is more than 20 then adding more animals will not increase the chance of getting significant results. In this case, the DF was kept at 35 and considered an adequate sample size that was applicable to this experiment.

3.9. Experimental design

The animals were placed into five groups (*n=7)* as follows:

I) Normal control group: received oral gavage of distilled water at 100µL daily

II) Diabetic untreated group: given High fructose diet in drinking water (HFD)

III) Diabetic + 100 mg/kg bw^{-1} metformin group: given HFD and treated with metformin orally (100 mg/kg bw^{-1})

IV) Diabetic + 300 mg/kg bw^{-1} extract group: given HFD and treated with TC orally (300 mgkg^{-1}bw^{-1})

V) Diabetic + 600 mg/kg bw^{-1} extract: given HFD and treated with TC orally (600 mgkg^{-1}bw^{-1}).

3.10. Animal treatment

Diets were provided for 15 weeks (12 weeks of T2DM induction followed by 3 weeks of treatment). During the entire treatment period, the rats were observed daily for clinical signs of toxicity on behavioral changes, morbidity and mortality. Fructose at 25% (w/v)

in drinking water was prepared daily by diluting 25 g of fructose with tap water to make 100 mL fructose solution in bottles; 500 mL of water or fructose solution were provided per cage. Aluminum foil was used to cover the bottles to avoid fructose fermentation. The specified doses of TC aqueous extracts and metformin at 100 mgkg^{-1}bw^{-1} were administered at 0800hrs orally once daily for 21 days post confirmation of diabetes status. Fasting blood sugar and body weights were determined at baseline and weekly. The weekly body weights were used to adjust treatment dosages accordingly so as to maintain the prescribed doses per body weights. The summary of the study design and treatment is as indicated in Appendix I.

3.11. Oral glucose tolerance test

This was done at the end of the treatment period (day 21) following 14 hours overnight fasting of the rats. 200 grams of glucose was dissolved in warm distilled water to make 1 litre of glucose solution and the rats were given 2 g glucose kg^{-1}bw^{-1} orally (Dupas *et al.*, 2016). Then appropriate amount of TC extract (300mg kg^{-1}bw^{-1} and 600 mgkg^{-1}bw^{-}), oral gavage of 100 µL distilled water to healthy control and standard drug (100mg kg^{-1}bw^{-1}) was then administered. The tail of the rats were sterilized using surgical spirit and blood was drawn from the tail vein before (0 min) and after 30, 60, 90, and 120 minutes after administration of glucose solution. Blood glucose levels were measured using glucometer as earlier described and the results were then recorded. Glucose tolerance was determined by plotting mean blood glucose values against time for each group.

3.12. Animal sacrifice, serum parameters analyses and tissue processing

At the end of the study and a day after performing OGTT, all the rats were fasted overnight (14 hours) and body weights measured then euthanized under mild anesthesia

of chloroform. The rats were then mounted on dissection bench and dissected using dissecting kit. Blood was collected through cardiac puncture and put in red top with clot activator vacutainer blood collection tubes. Skeletal muscle were carefully removed from the hind limb, washed with ice cold phosphate buffered saline (PBS), weighed using analytical balance (AUW220 Shimadzu corporation, Tokyo, Japan) and then immediately stored at -20˚C for biochemical assays.   The relative skeletal muscle tissue weight (ROW) were calculated and recorded in proportion to the body weight according to the following equation:

Relative organ weight (ROW) = <u>*Absolute organ weight*</u>   *X100*

Body weight of rat on day of sacrifice

Serum was obtained by centrifugation of whole blood collected in plain bottles at 3,000 x g for 20 minutes. Immediately after centrifugation, the serum samples were placed in an ice bath and kept at 4˚C. Fasting blood glucose, total cholesterol (T.CHOL), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), and triglycerides (TGs) were analyzed in serum. For renal function test, serum creatinine and urea were analyzed and for liver function indices, serum alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT), total proteins (TP), C-reactive protein (CRP) and albumin (ALB) were also analyzed. These tests were done using COBAS INTEGRA 400 plus auto-analyzer (Roche Diagnostic, Mannheim, Germany) at Moi Teaching and Referral Hospital laboratory, Eldoret, Kenya following manufacturer's instructions.

3.13. Analysis of triglyceride mass in skeletal muscle tissue

TG mass was measured as described by (Scribner *et al.*, 2000).. The frozen samples were thawed at room temperature for approximately 1 hour until completely thawed. 100 mg of frozen tissue was minced and homogenized in 2 mL of sucrose buffer (0.3 mol/L sucrose, 25 nmol/L 2-mercaptoethanol, and 10 mmol/L EDTA, pH 7.0). 200 µL aliquot of the suspension was transferred into 5 mL glass test tubes. 800 µL of chloroform was then added to each tube, mixed and allowed to stand for 30 min at room temperature. 250 µL of chloroform was added followed immediately by 250 µL of 0.15M NaCl and allowed to stand for 1 hour at room temperature. The mixture was then centrifuged at 1000rpm for 15 min. The lower organic phase was collected into clean labeled 5 mL tubes. The aqueous phase was then washed with 800 µL of chloroform to recover the lipids and centrifuged as above. The lower organic phase was collected and added to that separated from previous step. The combined organic phases were dried under a vacuum, and the lipids resuspended in 100 µL of 95% ethanol. 20 µL of the suspension were used for TG levels determination with a kit according to the manufacturer's instructions (Triglycerides GPO-PAP Method, Beacon Diagnostics Pvt. Ltd, Navsari, India). The rose colored dye produced during oxidative condensation of 4-Chlorophenol and 4-aminophenazone (4 AAP) was measured using spectrophotometer (Wagtech DU® 720, California, USA) at 550 nm. TG measurements (intensity of the colour formed being directly proportional to the triglycerides concentration in the sample) were then normalized to the weight of each tissue (milligrams of TG per gram of tissue).

3.14. Data management and statistical analysis

The data was entered into Microsoft office Excel and transferred to R software for statistical analysis. Statistical data analysis was by Tukey's test and one-way analysis of variance (ANOVA). Quantitative data were expressed as mean ± standard error mean (SEM). Values with $p < 0.05$ were considered to be statistically significant.

CHAPTER FOUR

RESULTS

4.1. Qualitative phytochemical evaluation of *Tarchonanthus camphoratus* crude aqueous leaf extract

 The qualitative phytochemical analysis of the TC crude aqueous leaf extract revealed the presence of flavonoids, saponins, phenolic compounds, terpenoids, tannins and steroids as shown in Table 4.1. Furthermore, alkaloids and cardiac glycosides were absent from the aqueous extract tested.

Table 4.1 Phytochemical constituents of aqueous *T. camphoratus* leaf extract

Phytochemicals	Presence (+) or absence (-)
Saponins	+
Tannins	+
Flavonoids	+
Terpenoids	+
Steroids	+
Cardiac glycosides	-
Phenolic compounds	+
Alkaloids	-

4.2 Clinical physical observations and body weight changes of rats on *T. camphoratus* leaf extract treatment

The physical observation of diabetic induced animals showed a reduction in physical activity than the normal control. The transient clinical signs that was most pronounced after dosing of rats and that lasted for about 30 min included raised fur and rubbing of oral cavity indicating irritation whenever the treatments were administered. Figure 4.1 shows that weight gain was similar in all diabetic rats over the 12-week fructose supplementation period ($p > 0.05$ versus initial weight, n=7 per group). Over the treatment period the diabetic untreated group had significantly higher weights compared to all other groups. Rats in the normal control group showed a significant lower mean body weights from day 0 to 21st day as compared to all the treatment groups ($^{*}p < 0.05$). The mean body weights in all the groups except diabetic + 600 mg/kg bw extract showed insignificant declining trend (7.8%) and had an overall trend of increase across the study period; however, the diabetic untreated group showed higher mean body weights (9.6% increase) with statistical significance of ($^{*}p < 0.05$) over the diabetic + 300 mg/kg bw extract treated and normal control groups (1.07% and 2.5% increases respectively).
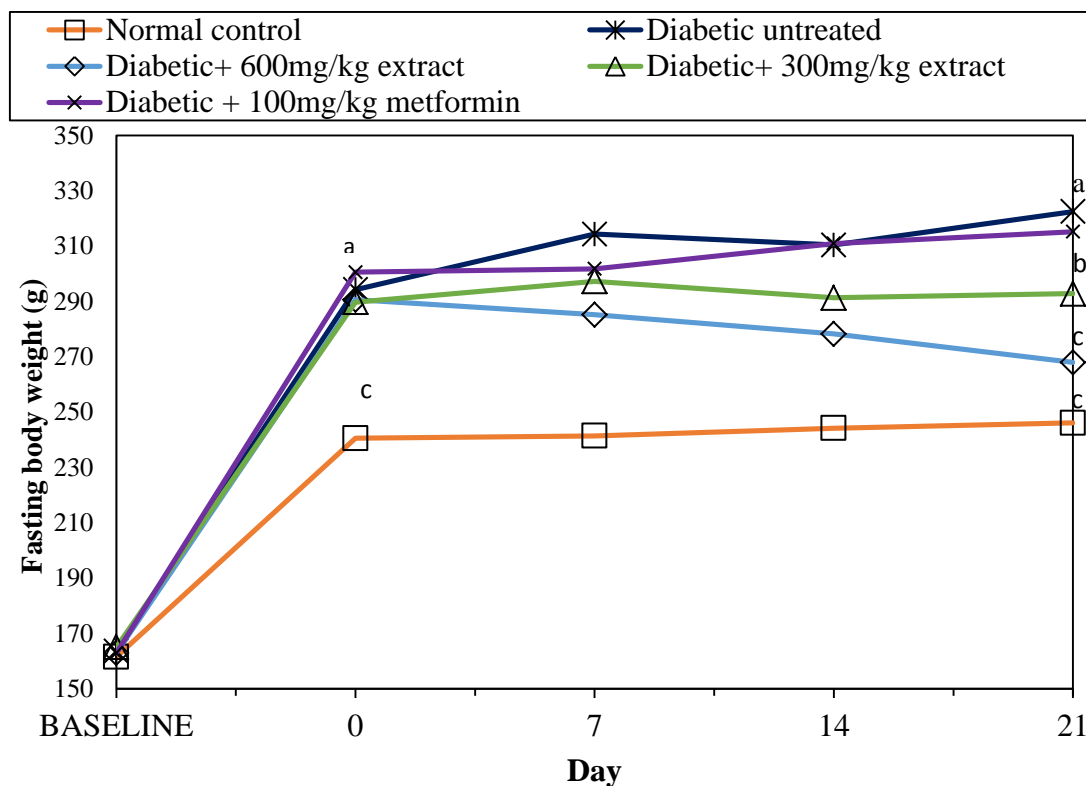
Figure 4.1 : Effects of *T. camphoratus* on fasting body weights of rats.

Aqueous leaf extracts at 300 mg/kgbw and 600 mg/kgbw on body weights of rats. Values represents the mean ± SEM; *n*=7. Superscript "a, b, c" Significantly different between the diabetic untreated and the normal control ($p < 0.05$, ANOVA).

4.3. Effect of *T. camphoratus* leaf extract on fasting blood glucose of rats

Before treatment (day 0), all the rats in the diabetic induced group had significantly higher levels of fasting blood glucose when compared to normal control (*$p < 0.05$). Diabetic untreated group had higher fasting blood glucose levels than normal control rats (6.30±0.28 vs. 3.76±0.28 mmol/L, *$p < 0.05$) as shown in Figure 4.2. However, treatment with 300 and 600 mg/kg bw of *T. camphoratus* leaf extract, and 100 mg/kg bw of metformin for 21 days, resulted in significant reduction in the mean fasting blood glucose

levels when compared with diabetic untreated group ($^*p < 0.05$) from day 0. The metformin 100 mg/kg bw treatment resulted in maximum reduction of 48.4% in fasting blood glucose, while 600 mg/kg bw and 300 mg/kg bw leaf extract treated groups exhibited a fall of 33.9% and 27.3% respectively as shown in figure 4.2.
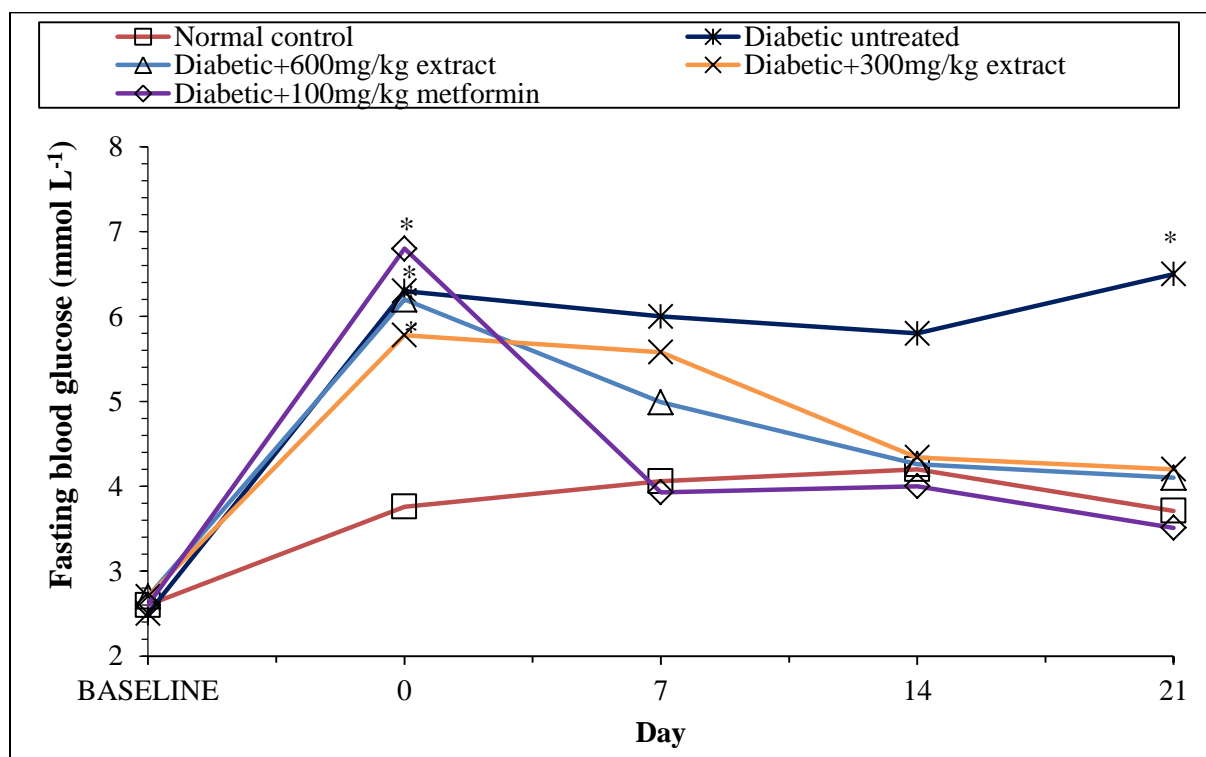
Figure 4.2: Effects of *T. camphoratus* on fasting blood glucose of rats.

Aqueous leaf extracts at 300 mg/kgbw and 600 mg/kgbw on fasting blood glucose of rats. Values represents the mean ± SEM; *n*=7. *Significantly different as compared to normal rats ($p < 0.05$).

4.4. Effects of *T. camphoratus* leaf extracts on glucose tolerance test of rats

Oral glucose tolerance test (OGTT) was performed at day 21 of treatment period. At age 21 weeks, OGTT results (Figure 4.3) showed that compared to the normal control rats, blood glucose levels of diabetic untreated group peaked at the 30th minutes and had a falling trend after the 60th min after the ingestion of a high dose of glucose (30 minutes: 8.67.±0.424 mmol/L vs. 7.83±0.424 mmol/L; 120 minutes: 5.77±0.424 vs. 4.40.6±0.424 mmol/L, both $^{*}p$ <0.05). Blood glucose for diabetic untreated group remained high at 120 minutes compared to normal control and other treatments and was highly significant ($^{*}p$ < 0.05). The blood glucose level in the normal control rats rose to the peak at 30 minutes after glucose load and decreased to near normal levels at 120 minutes. In the untreated diabetic rats, the peak increase in blood glucose concentration was observed after 30 minutes and remained high over the next 90 minutes.
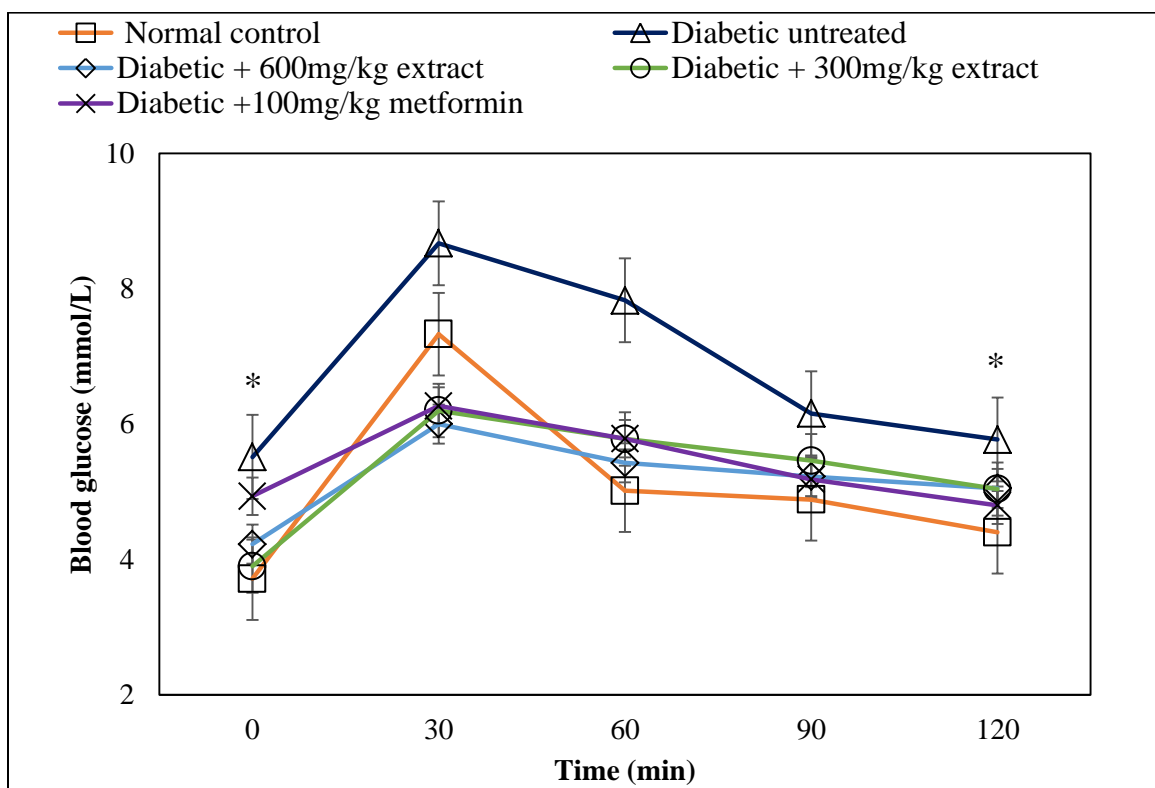
Figure 4.3: Effects of *T. camphoratus* on glucose tolerance.

Aqueous leaf extract on oral glucose tolerance test in rats. Data is expressed as the mean ± SEM (n=7). *$p < 0.05$ is significant difference in diabetic untreated group compared to normal and treated groups.

4.5. Effect of *T. camphoratus* leaf extract on serum lipid profile and indices of liver and kidney function of rats.

As shown in Table 4.2, the diabetic untreated rats exhibited a statistically significant ($p < 0.05$) elevated serum levels of ALT, ALP, and CRP as well as reduced TP and albumin levels when compared with the normal control and all other treatment groups. AST was insignificantly changed in all treatment groups compared to the normal control rats. A

significant increase in TP levels was recorded in the diabetic rats treated with 100 mg/kg bwt metformin compared with normal control rats ($p < 0.05$). The normal control and diabetic rats treated with 600 mg/kg bwt TC extract and 100 mg/kgbw metformin respectively recorded significant higher albumin levels than the diabetic untreated rats. The levels of ALT and ALP were significantly reduced when diabetic rats were treated with 300 and 600 mg/kgbw of *T. camphoratus* extract and 100 mg/kg bw of metformin as compared with the diabetic untreated rats. There was no significant change in all the serum kidney function indices (urea and creatinine) examined in all treatment groups when compared with the respective normal controls. There were no significant alterations in the levels of serum T.CHOL, LDL-C and HDL-C in the treatment and control groups. However, there was a significant elevation ($p < 0.05$) in the levels of serum triglycerides in diabetic untreated rats when compared with the normal control, TC 300 mg/kgbw, TC 600 mg/kgbw group and 100 mg/kgbw metformin treated groups. The aqueous extract of *T. camphoratus* significantly ameliorated ($p < 0.05$) the levels of serum triglycerides in the diabetic groups to near normalcy comparable to the values observed in the normal control after 21 days of treatment.

Table 4.2 Effect of *T. camphoratus* leaf extracts on serum lipid profile and indices of liver and kidney function of rats

Parameter	Sex (M-Male)	Normal Control	Diabetic untreated	Diabetic+300mg/kg extract	Diabetic +600mg/kg extract	Diabetic+100mg/kg metformin
ALT (U/L)	M ($n=7$)	64.00±4.53	92.10±4.53[*]	56.6±5.36[#]	64.20±6.00[#]	60.00±4.90[#]
ALP (U/L)	M ($n=7$)	95.90±25.93	206.22±21.24[*]	106.93±23.24[#]	121.52±25.91	95.70±21.22[#]
AST (U/L)	M ($n=7$)	128.00±15.92	147.12±15.90	112.00±18.80	124.00±21.00	115.00±17.20
CRP (mg/L)	M ($n=7$)	0.12±0.05	0.40±0.05[*]	0.60±0.06[*]	0.60±0.06[*]	0.60±0.05[*]
TP (g/L)	M ($n=7$)	65.44±0.87	65.94±0.87	67.22±1.03	68.80±1.15	69.20±0.87[*]
Albumin (g/L)	M ($n=7$)	37.70±1.02	33.30±1.02[*]	37.50±1.2	39.60±1.35[#]	40.10±1.10[#]
Creatinine(μmol/L)	M ($n=7$)	35.22±1.71	36.30±1.71	30.43±2.03	29.20±2.27	33.23±1.85
Urea (mmol/L)	M ($n=7$)	5.25±0.58	7.45±0.58	5.24±0.58	5.08±0.58	5.78±0.58
LDL-C (mmol/L)	M ($n=7$)	0.26±0.03	0.30±0.03	0.29±0.03	0.26±0.04	0.28±0.03
HDL-C (mmol/L)	M ($n=7$)	0.78±0.05	0.75±0.044	0.79±0.05	0.83±0.05	0.91±0.05
TG (mmol/L)	M ($n=7$)	0.55±0.04	0.82±0.04*	0.51±0.04[#]	0.49±0.04[#]	0.44±0.04[#]
T.CHOL (mmol/L)	M ($n=7$)	1.13±0.06	1.33±0.06	1.29±0.06	1.21±0.07	1.16±0.06

Values represent mean ± SEM; n=7. [*]Significant difference compared to normal control, [#]Significant difference compared with diabetic untreated group ($p < 0.05$, ANOVA). ALP (alkaline phosphatase), ALT (alanine aminotransferase), AST (aspartate aminotransferase), CRP (C-reactive protein), TP (total protein), LDL-C (low density lipoprotein cholesterol), HDL-C (high density lipoprotein cholesterol), TGs (triglycerides), T.CHOL (total cholesterol) and TC (*Tarchonanthus camphoratus*).

4.6 Effects of *T. camphoratus* leaf extract on relative weights and triglyceride mass of skeletal muscle of rats

As indicated in Table 4.3, the diabetic untreated group exhibited a statistically significant ($p < 0.05$) increase in TGM compared to normal control and also to the TC 300 mg/kg bw, TC 600 mg/kg bw and 100 mg/kg bw metformin treated groups. For relative skeletal muscle weight, we found an insignificant difference among treated groups although the diabetic untreated group had increased skeletal tissue weight compared with the normal control group.

Table 4.3 Effects of *T. camphoratus* leaf extract on relative weights and triglyceride mass of skeletal muscle of rats

Treatment group	Sex (M-male)	TGM (mg/g)	RTW (%)
Normal control	M (*n*=7)	1.69 ± 0.15^a	0.46 ± 0.01
Diabetic untreated	M (*n*=7)	3.37 ± 0.15^b	0.60 ± 0.09
Diabetic+300mg/kg TC	M (*n*=7)	2.35 ± 0.20^a	0.45 ± 0.01
Diabetic+600mg/kg TC	M (*n*=7)	2.22 ± 0.18^a	0.39 ± 0.01
Diabetic+ metformin (100mg/kg)	M (*n*=7)	2.12 ± 0.16^a	0.49 ± 0.00

Values are expressed as mean ± SEM. M, male; TGM, triglyceride mass (mg/g); RTW, relative tissue weight (%).Values are expressed as mean ± SEM (n=7). Superscript " a, b " across rows values are statistically significant at $p < 0.05$ in diabetic untreated group compared to normal and treated groups.

CHAPTER FIVE

DISCUSSION

T2DM is a multi-factorial disorder, usually associated with a set of metabolic syndromes such as obesity, hypertriglyceridemia, glucose intolerance and insulin resistance (IR) (Anvari *et al.*, 2014; Galicia-Garcia *et al.*, 2020). The potential of medicinal plants have been explored in the management (Ankita Singh *et al.*, 2018) and prevention of DM (Ekar *et al.*, 2019). This present study showed that *Tarchonanthus camphoratus* aqueous leaf extract is rich in phytochemicals including; tannins, saponins, flavanoids, terpenoids, phenolic compounds and steroids, whereas alkaloids and cardiac glycosides were not detected (Table 4.1).These findings are in agreement with (Ali *et al.*, 2013; Anvari *et al.*, 2014; Nanyonga *et al.*, 2013; van Huyssteen *et al.*, 2011) who reported the presence of saponins, tannins, flavonoids, phenolic compounds, terpenoids but not cardiac glycosides and alkaloids from their studies on TC leaf extract (Cock *et al.*, 2021).

Different approaches have been used to induce DM in experimental animals. In the present study, DM was induced using 25% fructose for twelve weeks. Rats in the normal control group showed a significantly lower mean body weights from day 0 to 21st day as compared to all the treatment groups ($^* p < 0.05$). The diabetic untreated rats in this study had significantly increased weights compared to all other groups. This could be attributed to increased energy intake due to fructose supplementation that induced metabolic syndrome markers (Di Luccia *et al.*, 2015). A fructose-rich diet in rats' increases abdominal adipocyte mass and impairs insulin sensitivity. It may also suppress food intake to a lesser extent than glucose and thus result in weight gain because fructose exhibited higher postprandial triglyceride levels and increased visceral adipose tissue

(VAT) compared with subjects fed glucose-sweetened drinks (Du *et al.*, 2012). The results of the present study are similar to those of (Brito *et al.*, 2008; Stranahan *et al.*, 2011) who demonstrated that high fructose diet promotes weight gain in rodents. High fructose consumption may results in an increase in body weight through the positive energy balance. Obesity due to positive energy balance is associated with a high concentration of free fatty acids which may diminish insulin sensitivity and thus increase in blood glucose level (Canfora *et al.*, 2015). The mean body weights in all the groups except diabetic + 600 mg/kg bw extract had an overall trend of increase across the study period; however, the diabetic untreated group showed a significantly increased mean body weights (9.6% increase) ($p < 0.05$) when compared to the diabetic + 300 mg/kgbw extract treated and normal control groups (1.07% and 2.5% increase respectively) (Figure 4.2). The declining trend in body weights of rats as observed in diabetic + 600 mg/kgbw *T.camphoratus*-treated rats (declined by 7.8% p>0.05) could be attributed to the extract's effects of increasing glucose utilization hence saving the body fat and muscle protein which contrarily are utilized in diabetic rats or owing to improved insulin and these findings are in agreement with (van Huyssteen *et al.*, 2011; Vedasree *et al.*, 2022) who demonstrated that aqueous extracts were more effective at increasing glucose utilization. The diabetic rats treated with TC leaf extract did not undergo extreme weight changes compared to untreated diabetic rats, a trend that was also observed with metformin treated group (Table 4.2). *Tarchonantus camphoratus* extract at 600 mg/kgbw was more effective in improving the mean body weights when compared to low dose extract of 300 mg/kgbw and metformin (100 mg/kgbw) that can be ascribed to its higher concentration of phytochemical components.

We also sought to determine the effects of *T. camphoratus* leaf extract on fasting blood glucose and compared also with metformin (100 mg/kgbw) as the standard drug. Upon treatment with 300 and 600 mg/kgbw of the extract for 21 days, there was significant improvement in the mean fasting blood glucose levels when compared with diabetic untreated group in both treatment groups. The metformin treated group resulted in maximum improvement by 48.4% in fasting blood glucose, while 600 mg/kgbw and 300 mg/kgbw leaf extract treated groups exhibited a dose dependent fall of 33.9% and 27.3% respectively. The hypoglycemic effect of *T. camphoratus* leaf extract might be due to the presence of various phytochemicals in TC including saponins, flavonoids, tannins, terpenoids, and phenolic compounds which have been shown to possess antihyperglycemic effects (Chauhan *et al.*, 2010). These observations are in agreement with previous studies by (Mukherjee *et al.*, 2006; Springfield *et al.*, 2005). Flavonoids have insulin-like effects and cause lipogenesis and glucose transport in the adipocytes thus reduction of blood glucose while terpenoids reduces diastolic blood pressure and lower the sugar level in blood (Arika *et al.*, 2015). Based on our findings, the reduction of blood sugar levels might have been brought about by the effects of TC extract on the activity of pancreatic ß cells of Langerhans to produce insulin or prevention of absorption of glucose from the intestine or may enhance the glucose uptake in peripheral tissues. Other mechanisms may involve increase in the protective/inhibitory effect against insulinase, increase hepatic glycogenesis and/or decrease of glycogenolysis acting on enzymes, reduction of glycogenic index of carbohydrates and of the crucial effect of glutathione in the maintenance of cellular redox homeostasis via detoxifying electrophiles. All of these actions may be responsible for the amelioration and or

management of diabetes mellitus and its complications (Al-Ishaq *et al.*, 2019; Deka *et al.*, 2021; Prabhakar *et al.*, 2011).

Glucose Tolerance Test (GTT) measures the body ability to utilize glucose, as the body's main source of energy for body cells (Sornalakshmi *et al.*, 2016). Oral glucose tolerance test (OGTT) was performed to assess the efficacy of antidiabetic effects of aqueous leaf extract of *T. camphoratus*. Increased fasting glycemia due to fructose supplementation is associated with reduced glucose tolerance. During glucose tolerance testing, the diabetic untreated group had higher plasma glucose levels compared to the other groups. This could be as a result of the establishment of insulin resistance (Rachel W Li *et al.*, 2006). These observations are similar to previous studies by (Do *et al.*, 2018) who revealed that fructose feeding leads to glucose intolerance. Glucose lowering effects observed in the extract treatments groups (diabetic + 300 mg/kg/bw and diabetic + 600 mg/kgbw) were comparable to those of metformin at 100 mg/kgbw. This means *T. camphoratus* aqueous leaf extracts promotes glucose tolerance. Although not investigated in the present study, we extrapolate that this is by either stimulation or regeneration of the pancreatic β cells for insulin secretion and are effective in controlling diabetes mellitus by improvement of carbohydrate metabolizing enzymes towards the re-establishment of normal blood sugar levels (Devi *et al.*, 2018).

No significant change was observed in the levels of serum T.CHOL, LDL-C and HDL-C examined in all treatment groups when compared with the normal control. However, there was a significant elevation ($p < 0.05$) in the levels of serum triglycerides in diabetic untreated rats when compared with the normal control, TC 300 mg/kg bw, TC 600 mg/kg bw and 100 mg/kg metformin treated groups. The aqueous extract of *T. camphoratus*

significantly improved the levels of serum triglycerides in the diabetic groups to near normalcy comparable to the values observed in the normal control. Dyslipidemia is a common characteristic observed in T2DM marked by serum hypertriglyceridemia, increased LDL-C and reduced levels of HDL-C. It is also directly linked to IR as high circulating lipid concentrations in the blood secrete humoral factors such as resistin and adiponectin that alter insulin sensitivity, leading to IR (Echegoyen *et al.*, 2023; Wilson *et al.*, 2012; Yanai *et al.*, 2019). In the present study, there were no significant alterations in the levels of serum T.CHOL, LDL-C and HDL-C in the treatment and control groups suggesting that *T.camphoratus* leaf extract did not affect these parameters. However, there was a significant elevation in the levels of serum triglycerides in diabetic untreated rats when compared with the normal control and other treated groups. This suggests that fructose successfully induced hypertriglyceridemia through either liver injury marked with an increase in lipogenesis or impaired carbohydrate metabolism that resulted in insulin resistance and augmented TG serum levels. This is partly because fructose metabolism cannot be controlled by insulin or leptin, which are important factors for the regulation of fat synthesis as well as energy intake. Several recent studies have reported that higher serum triglycerides alone or accompanied with low serum HDL-cholesterol is a risk factor for hepatic IR as well as T2DM (Milutinović *et al.*, 2020). The aqueous extract of *Tarchonanthus. camphoratus* ameliorated the levels of serum triglycerides in the diabetic treated groups to near normalcy comparable to the values observed in the normal control group and this finding can be attributed to extract's prevention effects of triglycerides elevation (Cock *et al.*, 2021).

Skeletal muscle is a major site of insulin-mediated glucose disposal where the fatty acid composition of skeletal-muscle phospholipids is closely related to insulin resistance (Rachek, 2014). Increased fructose intake has been shown to impair glucose utilization associated with induction of oxidative stress in clonal skeletal muscle cells (Chadt *et al.*, 2020). It also impairs normal lipid and carbohydrate metabolism and facilitates the non-enzymatic glycation reaction leading to enhanced accumulation of advanced glycation end products (AGEs) in skeletal muscle hence impaired glucose homeostasis (Dozio *et al.*, 2021). As shown in Table 4.3, there was a significant increase in triglyceride flow to skeletal muscle in diabetic untreated rats compared to normal and treated groups and a significant increase in relative skeletal muscle weight when compared with the normal control. The findings in the present study suggest that high-fructose diet even without polyphagia is able to increase lipid flow to skeletal muscle and mitochondrial energetic efficiency, with two hypothesized deleterious effects: (i) energy sparing that contributes to the early onset of obesity and (ii) reduced oxidation of fatty acids and lipid accumulation in skeletal muscle, which could generate insulin resistance (Oke, 2021). We also sought to determine whether *T camphoratus* crude extract would improve skeletal muscle tissue weight. The extract treated groups exhibited significant decrease in relative skeletal muscle weight when compared with the diabetic untreated rats; this might have been brought about by the bioactive components present in TC leaf extracts that prevents adiposity and alleviate insulin resistance (Forney *et al.*, 2018; Guru *et al.*, 2021). These findings are in agreement with (Nimrouzi *et al.*, 2020; Sathyanarayana *et al.*, 2022) who reported more decreased tissue weights that was observed with increasing amounts of tannins, phenols and flavonoids. In our study, the lowest TG mass level and tissue weight

gain was observed in diabetic rats receiving 600 mg/kg extract group confirming results of other investigators. It can be hypothesized that the changes in body and relative tissue weight seemed to be markedly influenced by the mean change in triglyceride mass and/or decreased dietary food intake.

Liver is one of the most vital body organ plays a key role in metabolism of endogenous substances and drugs. The dysfunction of this organ cause changes in its biochemical indices such as ALT, ALP and AST that might be due to outflow of these enzymes from liver's cell cytoplasm to the blood stream that determined the extent of hepatocyte injury. Diabetes mellitus is one of the metabolic syndromes associated with elevation of these liver marker enzymes (Küçükler *et al.*, 2021; Napierala *et al.*, 2019). In this study, the diabetic untreated rats exhibited significantly elevated serum levels of ALP, ALT and CRP and reduction in total proteins and albumin levels when compared with the normal control and all other treatment groups. Elevated ALP, ALT, CRP and a reduction of TP and albumin levels could be as a result of damaged hepatocytes due to fructose-induced hyperglycemia (Falode *et al.*, 2023; Schumacher-Petersen *et al.*, 2019). The blood biomarker, CRP is thought to be a useful marker during an acute inflammatory assault (Saklani *et al.*, 2016). It has been demonstrated that an increase in baseline CRP levels can be used to assess liver damage brought on by chronic inflammation, excessive inflammation or a failure of the first inflammatory response (Furman *et al.*, 2019). On the other hand, there was significant reduction of ALT, ALP levels in diabetic rats treated with 300 and 600 mg/kg bw of *Tarchonanthus camphoratus* extracts and 100 mg/kg bw of metformin compared with the diabetic untreated rats. This could be attributed to nontoxic nature and protective action of the extract in reversing liver damage due to

diabetes mellitus (Arya *et al.*, 2015; Sunmonu *et al.*, 2013). Interestingly, significant increase in TP levels was recorded in the diabetic rats treated with 100 mg/kg bwt metformin compared with normal control rats. The normal control and diabetic rats treated with 600 mg/kg bwt TC extract and 100 mg/kg bw metformin respectively recorded significant higher albumin levels than the diabetic untreated rats. Therefore, the increase in serum albumin and total protein levels after TC extract administration to the rats restored the albumin and TP levels to normalcy. This further confirmed bestowment of protection of *T. camphoratus* bioactive components to the liver of diabetic rats. In this study, renal function was not affected as evident by insignificant change of serum creatinine and urea. Further studies on detailed mechanistic anti-diabetic properties of the crude leaf extract e.g., on gene expression studies and *in vivo* anti-oxidant properties can provide more information as this was a limitation in this study.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Fructose supplementation (25% w/v for 12 weeks) in Wistar albino male rats induced progressive development of some metabolic syndrome markers (hyperglycemia, increased bodyweight and hypertriglyceridemia) as well as insulin resistance. *Tarchonanthus camphoratus* aqueous leaf extract is rich in phytochemicals that include saponins, tannins, flavonoids, terpenoids and phenolic compounds that proofed to possess health benefits, including a role in the treatment of hyperlipidemia and diabetes mellitus management in diabetic rats. The 3-week oral administration of *T. camphoratus* aqueous leaf extract in diabetic rats exhibited significant amelioration of body weights and blood glucose levels. *Tarchonanthus camphoratus* extracts promoted glucose tolerance and also the results revealed significantly reduced serum ALT, ALP, CRP and TG levels in diabetic rats and this may be indicative of the nontoxic nature of the extract hence hepatoprotective properties and improvement in the imbalance in lipid metabolism experienced during diabetes mellitus. Further, in this diabetic rat model we have provided evidence that the 15- week high fructose diet supplementation and 300 mg/kg and 600 mg/kg TC extract treatment did not compromise the renal functions. *Tarchonanthus camphoratus* crude leaf extract therefore possesses potential for alternative medicine for diabetes mellitus treatment and management.

6.2 Recommendations

This study validates the herbal claims of *Tarchonanthus camphoratus* leaves potential for diabetes mellitus management. The following recommendations are put forth;

(i)   *Tarchonanthus camphoratus* is safe and efficacious for diabetes mellitus treatment and it is therefore recommended for continued use as herbal remedy for diabetes mellitus.

(ii)   Further investigations are needed to identify the specific antidiabetic compounds in *Tarchonanthus camphoratus* leaf extract and to elucidate their mechanisms of action.

(iii)   Toxicity studies need to be undertaken to ascertain the safety of the *Tarchonanthus camphoratus* leaf extract.

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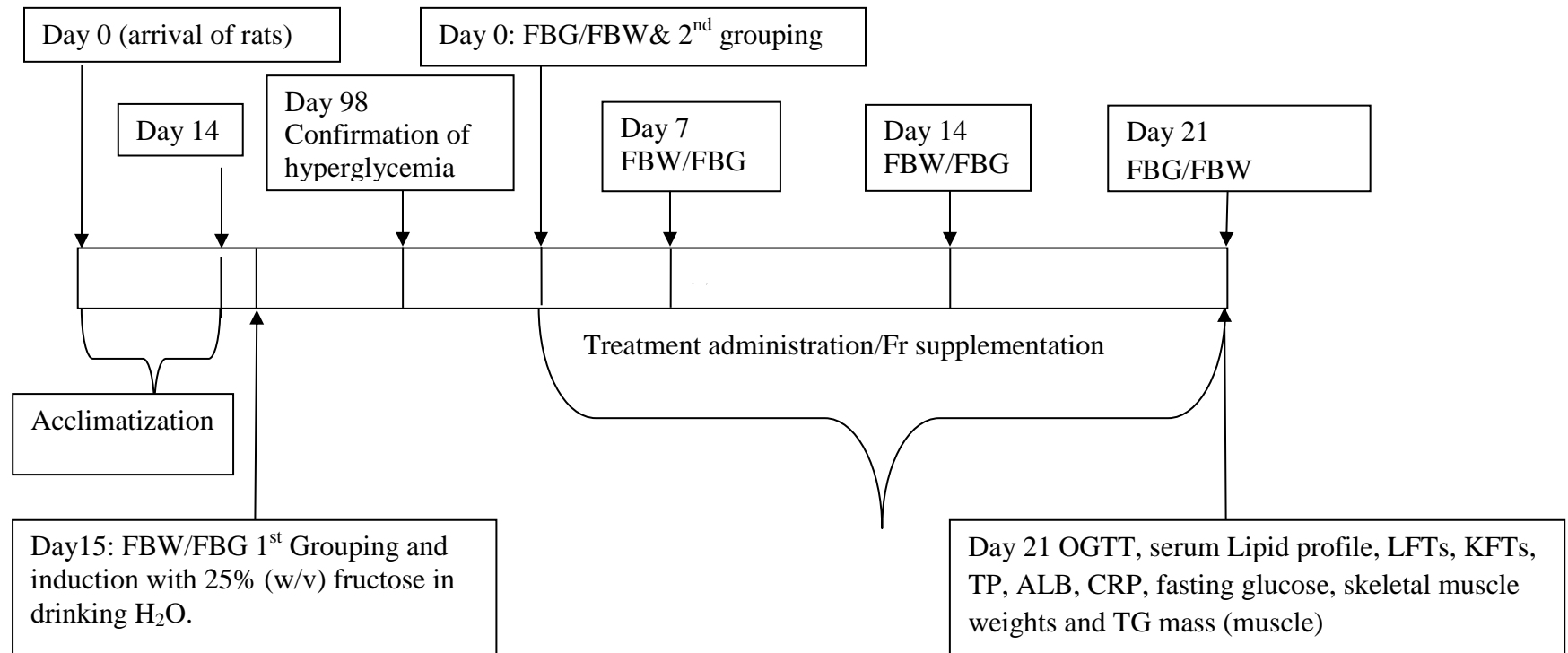Subramanian, & Baidal. (2021). The management of type 1 diabetes. *Endotext [Internet]*.

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APPENDICES

Appendix I: Summary of the Study Design and Treatment

| Day 0 (arrival of rats) | Day 0: FBG/FBW& 2nd grouping |

| Day 14 | Day 98 Confirmation of hyperglycemia | Day 7 FBW/FBG | Day 14 FBW/FBG | Day 21 FBG/FBW |

Treatment administration/Fr supplementation

Acclimatization

Day15: FBW/FBG 1st Grouping and induction with 25% (w/v) fructose in drinking H$_2$O.

Day 21 OGTT, serum Lipid profile, LFTs, KFTs, TP, ALB, CRP, fasting glucose, skeletal muscle weights and TG mass (muscle)

Key

FBG-Fasting blood glucose   TP-Total protein

FBW-Fasting body weight   ALB-Albumin

OGTT-Oral glucose tolerance test   CRP- C reactive protein

LFTs-Liver function test   TG- Triglyceride

KFTs-Kidney function test

Appendix II: Research Ethical Clearance Letter

OFFICE OF THE DIRECTOR OF GRADUATE STUDIES
AND RESEARCH
UNIVERSITY OF EASTERN AFRICA, BARATON
P. O. Box 2500-30100, Eldoret, Kenya, East Africa

March 24, 2017

Ngeno Bernard Kiprotich
University of Eldoret
School of Science

Dear Ngeno,

Re: ETHICS CLEARANCE FOR RESEARCH PROPOSAL (REC: UEAB/6/3/2017)

Your research proposal entitled *"Evaluation of Antidiabetic Properties of Tarchonanthus camphoratus in Induced Diabetic Wistar Rats"* was discussed by the Research Ethics Committee (REC) of the University and your request for ethics clearance was granted approval.

This approval is for one year effective March 24, 2017 until March 24, 2018. For any extension beyond this time period, you will need to apply to this committee one month prior to expiry date. Note that you will need a clearance from the study site before you start gathering your data.

We wish you success in your research.

Sincerely yours,

Dr. Jackie K. Obey
Chairperson, Research Ethics Committee

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Appendix III: Similarity Report

University of Eldoret
Certificate of Plagiarism Check for Synopsis

Author Name	Benard Kiprotich Ngeno SC/PGC/012/13
Course of Study	Type here...
Name of Guide	Type here...
Department	Type here...
Acceptable Maximum Limit	Type here...
Submitted By	titustoo@uoeld.ac.ke
Paper Title	ANTIDIABETIC PROPERTIES OF Tarchonanthus camphoratus IN FRUCTOSE-INDUCED DIABETIC WISTAR RATS
Similarity	11%
Paper ID	994041
Submission Date	2023-10-02 09:29:29

Signature of Student   Head of the Department   Signature of Guide

University of Eldoret
P.O. Box 1125, ELD

* This report has been generated by DrillBit Anti-Plagiarism Software