EFFECTS OF NON-BITTER *CUCUMIS METULIFERUS* (KIWANO) FRUIT EXTRACT ON THE METABOLIC PROFILES OF STREPTOZOTOCIN-INDUCED TYPE II DIABETIC WISTAR ALBINO RATS

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Effects of Non-Bitter *Cucumis Metuliferus* (Kiwano) Fruit Extract on the Metabolic Profiles of Streptozotocin-Induced Type II Diabetic Wistar Albino Rats

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

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DEDICATION

I dedicate this work to my family, relatives, and friends for their support and encouragement. Your being there for me in deed and in words, even when things looked tough, made me strong and gave me the energy I needed to complete this project. Thank you so much, and may God bless you all.

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ACRONYMS AND ABBREVIATIONS

ALB	Albumin
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate Aminotransferase
CMFE	*Cucumis metuliferus* Fruit Extract
CO2	Carbon Dioxide
DBIL	Direct Bilirubin
DKA	Diabetic Keto-Acidosis
DM	Diabetes Mellitus
DMSO	Dimethyl Sulfoxide
DPX	Distyrene, Plasticizer, and Xylene
EDTA	Ethylene-Diamine-Tetra-acetic Acid
FBS	Fasting Blood Sugar
GGT	Gamma Glutamyl Amino-Transferase
GLUT 2	Glucose Transporter 2
Hb A_{1c}	Haemoglobin A_{1c}

HDL	High density Lipoprotein
HHS	Hyperosmolar Hyperglycaemic State
JKUAT	Jomo Kenyatta University of Agriculture and Technology
JPEG	Joint Photography Experts Group
LDL	Low Density Lipoprotein
LD_{50}	Lethal Dose 50
NaCL	Sodium Chloride
OECD	Organization for Economic Cooperation and Development
OGTT	Oral Glucose Tolerance Test
PGZ	Pioglitazone
SAFARI	Small Animal Facility for Research and Innovation
SoMED	School of Medicine
SoPH	School of Public Health
SPSS	Statistical Package for the Social Sciences
STZ	Streptozotocin
TBIL	Total Bilirubin
TC	Total Cholesterol
TG	Triglyceride

TIF	Tagged Image Format
TP	Total Protein
USA	United States of America
USD	United States Dollar

DEFINITION OF OPERATIONAL TERMS

Acute oral toxicity This refers to the adverse effects occurring after an oral administration of a single dose of a substance or multiple doses given within 24 hours

Cucumis metuliferus This is a herbal plant that belongs to the cucumber and melon families. The fruit, known as horned melon/thorn melon in English or Kiwano in Kenyan, is usually eaten raw to treat diabetes mellitus in local communities like Kikuyu.

Metabolic profiles This entails the following: Blood sugar level (indicators: fasting blood sugar, oral glucose tolerance test, and haemoglobin A_{1c}) and liver functions (indicators: liver enzymes, serum proteins, serum bilirubin, and lipid profile)

Streptozotocin A naturally occurring alkylating antineoplastic agent toxic to the insulin-producing beta cells of the pancreas in mammals.

ABSTRACT

Globally, about 400 million people have diabetes mellitus (DM), of whom the majority live in low- and middle-income countries. Every year, about 1.5 million deaths are directly attributed to diabetes mellitus and its associated complications. The cost of management of diabetes mellitus and its related complications is beyond reach to many people in low- and middle-income countries, necessitating the evaluation of locally available treatment alternatives. Although existing literature suggests that *Cucumis metuliferus* fruit possesses hypoglycaemic properties, empirical data to support this claim is scanty. The main objective of this study was to determine the effects of non-bitter *Cucumis metuliferus* fruit extract (CMFE) on the metabolic profiles of streptozotocin-induced type II diabetic Wistar Albino Rats. A total of 64 male Wistar albino rats weighing between 90 and 130 grams and aged 5 weeks were randomly assigned into two major groups, i.e., the control group and the experimental group. The control group received a standard rodent pellet diet plus 0.9% normal saline, whereas the experimental group received a high-fat/fructose diet plus streptozotocin (STZ) injection to induce diabetes mellitus. The experimental group was further divided into a positive control group, which was treated with pioglitazone (the standard drug) at a dose of 20 mg/kg body weight, a low-dose CMFE group (200 mg/kg body weight), and a high-dose CMFE group (400 mg/kg body weight). Fasting blood sugar (FBS), the oral glucose tolerance test (OGTT), and HbA1C were used as indicators of blood sugar. Serum HDL, LDL, total cholesterol, triglycerides, AST, ALT, GGT, ALP, total bilirubin, direct bilirubin, albumin, and total protein were used as indicators for liver functions. Body weight was recorded weekly, and at the endpoint of each group, the rats were fasted for 6–8 hours, anaesthetized with CO2, and fresh blood samples were collected through intra-cardiac puncture. "The ACCU CHEK" glucometer was used to test for FBS and OGTT, while the Clover A_{1C} Self Hb A_{1C} Analyser was used to test for Hb A_{1C}. A CS T240 auto-chemistry analyser was used to test for liver functions. The pancreas was harvested for histological examination. The experiment ran for 70 days. The data was entered into a Microsoft Excel Spreadsheet and then transferred to SPSS version 25 for analysis. Comparison of multiple means was done using ANOVA, and Tukey's statistical test was used for Post hoc analysis. The analysis was done at a 95% level of confidence ($P = 0.05$). The data was presented using tables and figures. The study findings established that the extract contains glycosides, alkaloids, saponins, and tannins. An acute oral toxicity study revealed that at doses of 50 mg/kg, 300 mg/kg, and 2000 mg/kg body weight, the non-bitter CMFE is safe when administered to rats. This study established that treatment with a high-fat/fructose diet to induce type II DM significantly raised FBS ($P < 0.001$) and OGTT ($P < 0.001$), followed by a decrease to levels comparable to the control group after treatment with the non-bitter CMFE. Consequently, there was no significant difference ($P > 0.05$) in Hb A_{1c} test results, indicating that treatment with CMFE had a long-term control effect on blood sugar. Similarly, treatment with a high-fat/fructose diet significantly increased serum total cholesterol (TC) ($P < 0.001$), triglycerides (TGs) ($P = 0.019$), and low-density lipoproteins (LDL) ($P = 0.016$), followed by a decrease to levels comparable to the control group after treatment with the non-bitter CMFE. On the contrary, treatment with a high-fat/fructose diet significantly decreased ($P = 0.004$) serum high-density

lipoproteins (HDL), which was followed by a significant increase (P =0.001) after treatment with non-bitter CMFE. The findings also established that treatment with streptozotocin to induce type II DM significantly increased serum alanine aminotransferase (ALT) (P <0.001), aspartate aminotransferase (AST) (P <0.001), and gamma-glutamyl transferase (GGT) (P =0.002), followed by a decrease to levels comparable to the control group after treatment with the non-bitter CMFE. Notably, treatment with streptozotocin (STZ) to induce type 2 DM markedly reduced cell density in the Islet of Langerhans, indicative of STZ-induced beta cell dysfunction. However, treatment with CMFE increased the number of metabolically active cells in the Islet of Langerhans. In conclusion, this study demonstrates that CMFE has hypoglycaemic, hypolipidemic, and hepato-restorative properties. The study recommends the use of the non-bitter *Cucumis metuliferus* fruit extract as an adjunct in the control of blood sugar in type II DM. The study also recommends the use of the non-bitter *Cucumis metuliferus* fruit extract in the management of diabetic and nutritionally induced dyslipidemias.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (Karalliedde & Gnudi, 2016). Type II diabetes mellitus is a metabolic disorder characterised by insulin resistance and insulin deficiency, which leads to high glucose levels in the blood (Galicia *et al*., 2020). High blood glucose levels in turn damage many of the body's systems, particularly blood vessels and nerves (Banday *et al*., 2020). Such damage to the body systems is caused by disturbances in the regulatory systems responsible for the storage and utilization of the chemical energy from food. This includes the metabolism of carbohydrates, fats, and proteins resulting from defects in insulin secretion, insulin action, or both (Piero, 2015).

According to Nepalia (2017), poor glycaemic control and dyslipidaemia are associated with an increased incidence of diabetic complications like neuropathy, retinopathy, and nephropathy. Dyslipidemia, hypertension, and atherosclerosis, which are complications of diabetes mellitus, have been known to increase the risk of coronary heart disease, a leading killer disease in the world (Al-Nozha *et al*., 2016). Cardiovascular diseases that occur in patients with either type 1 or type 2 diabetes are associated with dyslipidemia-linked atherosclerosis. Acute metabolic complications of diabetes mellitus include diabetic ketoacidosis (DKA) and hyperosmolar hyperglycemic state (HHS). Due to the decreased ratio of insulin to glucagon, gluconeogenesis, glycogenolysis, ketone body formation in the liver, and an increase in free fatty acid and amino acid delivery from fat and muscle to the liver are promoted (Srivastava, 2014).

The global burden of diabetes is increasing worldwide as it is a costly disease for developing economies around the world (Ozougwu, 2013). About 3.5 % to 5 % of the Kenyan population are diabetic (Mohamed *et al*., 2018). The high prevalence of poor

diabetic control in children and adolescents in Kenya has predisposed them to the early development of microvascular complications. Adolescents with type 1 DM have particularly poor blood glucose control, making them a special high-risk group (Ngwiri *et al.*, 2015). According to Jones (2013), Kenya is still facing a challenge in tackling the burden of diabetes mellitus because of a lack of funds to effectively implement strategies geared towards its detection, management, and prevention.

In present times, researchers in pharmacology are increasingly studying medicinal plants across the globe. According to the World Health Organization (WHO), more than 80% of people across the globe do not seek conventional medicine for their primary health care services but rather rely on traditional medicine, where the majority use plants or their products (Ekor 2014). In Africa, several plants are used for the treatment of various human and animal diseases. These diseases include but are not limited to, diarrhoea, tuberculosis, diabetes mellitus, and skin diseases. Reports of various plants used in the treatment of diseases have been documented in countries like Nigeria (Sofowora *et al*., 2013), Kenya (Matu *et al*., 2012), Ethiopia (Gedif & Hahn, 2003), Turkey, Italy, and Panama, among others. These herbal options may be the only alternative in places where modern medical care services are not available or affordable. Treatment failure due to microbial resistance to commercial drugs has also necessitated the search for new anti-microbial substances from other sources, like plants. *Cucumis Metuliferus* and other plants of the family Cucurbitaceae have been documented as some of the plants of medicinal value (Usman *et al*., 2018).

The prevalence of traditional medicine use in the management of diabetes in Africa ranges between 12.4% and 77.1%, and 35.4% to 88.4% use traditional medicine concurrently with conventional medicine (Ekpor *et al*., 2023). However, many rural communities in Africa do not see any dangers associated with the use of herbal medicine, which is contrary to the beliefs of modern medical practitioners (Okaiyeto & Oguntibeju, 2021). In Kenya, many people in rural settings depend on traditional healers for certain ailments when access to specialist health practitioners is restricted or the cost of conventional medicine is not affordable (Lambert *et al*., 2011). In a study conducted by Mwangi and Gitonga

(2014), 12.4% of diabetic patients confessed to using herbal remedies as part of their management of diabetes. While conventional medicine is available, traditional medicine may provide affordable and effective care for some ailments. Indeed, this may have played a role in the move made by the Kenyan Government to try and incorporate some useful aspects of traditional medicine into the national medical health system (Chebii *et al*., 2020).

Cucumis metuliferus is a fruit belonging to the Cucurbitaceae family. It has a yellow-orange exterior when ripe and a green exterior when unripe, with thick, blunt spines. Inside, there are seeds embedded in a green, gelatinous pulp. It is native to Central and South Africa, although it is now grown all over the world. Provided the right temperature, it is an annual vine that can grow at any time. The phytochemicals present in Cucumis metuliferus are saponins, tannins, alkaloids, and glycosides (Dhale, 2011). These phytochemicals are effective against some illnesses caused by viruses, fungi, and bacteria. They also have analgesic, antimalarial, antidiabetic, antitumor, and diuretic activities (Kwaghe *et al*., 2015). A study on *Cucumis metuliferus* fruit extract (CMFE) demonstrated hypoglycemic activity in experimental rats (Jimam *et al*., 2010). However, scientific data to substantiate the fruit's therapeutic claim and effect on other metabolic profiles is scanty.

1.2 Statement of the Problem

Diabetes mellitus continues to be an increasing international health burden, with ageing and urbanization increasingly adding to its burden in developing countries, where resources for dealing with the associated clinical problems are most scarce (Animaw & Seyoum, 2017). The prevalence of diabetes in the world is projected to increase from 8.8% in 2015 to 10.0% in 2030, the number of diabetes-related deaths from 3.1 million in 2015 to 4.2 million in 2030, and the total absolute costs incurred in the management of DM and its associated complications will rise from 1.32 trillion USD in 2015 to 2.12 trillion USD in 2030 (Bommer *et al*., 2018). The average annual cost of managing diabetes and its related complications per patient in Kenya is Kshs 23,174 (Oyando *et al*., 2020). In Kenya, there is inadequate funding for the effective implementation of an effective

strategy for the prevention, detection, and management of diabetes (Jones, 2013). The Kenyan population that lives below the poverty line is quite high, and traditional herbal medicine may have a big role to play in the treatment of diabetes mellitus and its associated complications (Lambert *et al*., 2011). Although herbal plants are widely used and considered relatively safe in countries like Kenya and Sub-Saharan Africa in general, the risk of toxicity is high, hence the need to establish their safety profiles (Boukandou, 2015). The empirical data on the effects of CMFE on the metabolic profiles of type II diabetes mellitus is scant.

1.3 Justification of the Study

Utilization of an animal model in this study served as a "pre-trial" for the possible development of a drug from *Cucumis metuliferus*. The study findings may inform traditional healers and general population about the effectiveness of CMFE as an anti-diabetic agent and this could lower the cost of management of type 2 DM since *Cucumis metuliferus fruit* is locally grown and cheaply available in markets across the country. This study forms a foundation for researchers wishing to carry out similar or related studies in the future. Pharmaceutical companies wishing to isolate active ingredients in CMFE may benefit from the findings of this study, which may help in the large-scale production and commercialization of the fruit and its products. Discoveries may trigger the need to obtain patents.

1.4 Research Questions

1. What are the phytochemicals present in the non-bitter *Cucumis metuliferus* fruit extract?
2. What is the acute oral toxicity profile of the non-bitter *Cucumis metuliferus* fruit extract in Wistar albino rats?
3. What are the effects of non-bitter *Cucumis metuliferus* fruit extract on fasting blood sugar, oral glucose tolerance test, and haemoglobin A_{1C} of Streptozotocin-induced type II diabetic Wistar Albino rats?

4. What are the effects of non-bitter *Cucumis metuliferus* fruit extract on the liver functions of Streptozotocin-induced type II diabetic Wistar albino rats?
5. What are the effects of non-bitter *Cucumis metuliferus* fruit extract on the histomorphology of Islets of Langerhans in streptozotocin-induced type II diabetic Wistar Albino rats?

1.5 Objectives of the study

1.5.1 Broad Objective

To determine the effects of non-bitter *Cucumis metuliferus* (Kiwano) fruit extract on the metabolic profiles of streptozotocin-induced type II diabetic Wistar albino rats.

1.5.2 Specific Objectives

1. To determine the phytochemicals present in non-bitter *Cucumis metuliferus* fruit extract
2. To determine the acute oral toxicity profile of the non-bitter *Cucumis metuliferus* fruit extract in Wistar albino rats.
3. To determine the effects of non-bitter *Cucumis metuliferus* fruit extract on fasting blood sugar, oral glucose tolerance test, and haemoglobin A_{1C} of Streptozotocin-induced type II diabetic Wistar albino rats.
4. To determine the effects of non-bitter *Cucumis metuliferus* fruit extract on the liver functions of Streptozotocin-induced type II diabetic Wistar albino rats.
5. To determine the effects of non-bitter *Cucumis metuliferus* fruit extract on the histomorphology of Islets of Langerhans in streptozotocin-induced type II diabetic Wistar albino rats**.**

1.6 Hypothesis

H_0: *Cucumis metuliferus* fruit extract has no effects on the metabolic profiles of Streptozotocin-induced type II diabetic Wistar Albino rats.

$H_{A:}$ *Cucumis metuliferus* fruit extract affects the metabolic profiles of Streptozotocin-induced type II diabetic Wistar Albino rats.

1.7 Theoretical Framework

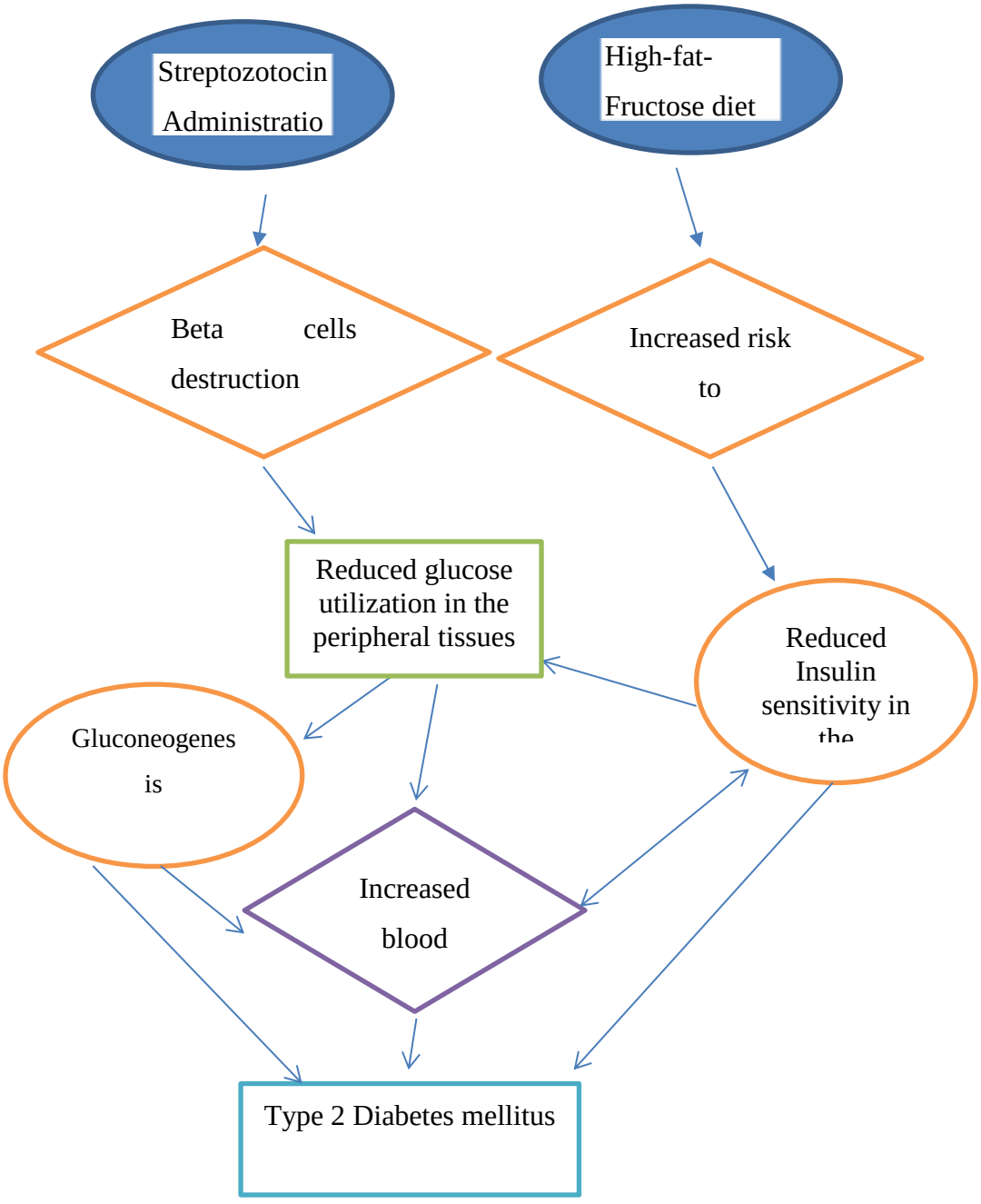

Figure 1.1: Theoretical Framework

CHAPTER TWO

LITERATURE REVIEW

2.1 *Cucumis metuliferus*

2.1.1 Brief Description of *Cucumis metuliferus*

The Cucumis metuliferus (horned melon) plant is an annual, mostly climbing herb, sometimes a creeper, with a trailing, 5-metre-long stem that radiates from a woody rootstock. Tendrils are slender and simple. Leaves are ovate or cordate in outline with a large basal sinus. Flowers are monoecious and funnel-shaped. The fruit is ellipsoid and cylindrical, with obscurely trigonous scattered spines carried on a 20–70 mm-long peduncle. Its colour is deep green, ripening yellow to orange-red, with longitudinal bands of pale markings. A fruit has hundreds of seeds that are ellipsoid and flattened and embedded in a light green, emerald-green, or translucent, jelly-like flesh (Mey, 2016).

Figure 2.1: *Cucumis metuliferus* vine and Its Fruits

Source: (Mey, 2016)

2.1.2 Scientific Profile of *Cucumis metuliferus*

There are two types of *Cucumis metuliferus*: the bitter and the non-bitter forms. The bitter form is toxic to both humans and animals and is therefore not palatable. The non-bitter form is edible and of medicinal value (Kwaghe *et al*., 2015; Usman *et al*., 2015). Non-bitter CMFE has been shown to contain metabolites such as alkaloids, carbohydrates, cardiac glycosides, flavonoids, saponins, tannins, steroids, and terpenoids (Usman *et al., 2015*). Dhale (2011) did a phytochemical screening of the fruit extract and found that saponins, tannins, alkaloids, and glycosides were present. These phytochemicals have antiviral, antifungal, antimicrobial, antitumor, antidiabetic, antimalarial, diuretic, and analgesic effects (*Uses of Cucumis Metuliferus : A Review*, 2015). According to Abubakar *et al*. (2011), the fruit extract also showed anti-trypanosomal activity when used against *Trypanosoma brucei* infection. Wannang *et al*. (2008) also demonstrated the anti-ulcer activity of the fruit extract in experimental rats.

2.1.3 Taxonomical Classification of *Cucumis metuliferus*

Kingdom -Plantae
Subkingdom - Tracheobionta
Superdivision -Spermatophyta
Division - Magnoliophyta
Class - Magnoliopsida
Subclass - Dilleniidae
Order - Violales
Family - Cucurbitaceae
Genus - *Cucumis* L.
Species - *Cucumis metuliferus* E. Mey. ex Naud.

Source: (Kotschy, 2015)

2.1.4 Common Names of Non-Bitter *Cucumis metuliferus*

English (Thorny melon, African horny melon, African cucumber, spiny cucumber, jelly melon, Kiwano, horny cucumber), Afrikaans (Doring komkommer), Finish (Sarvimelon, Kiwano, Kivakurkku), Shona (mushonja, mutate, mugamgangam, mugagachiga, mugaka), and Kenya (Kiwano) (Dhale, 2011).

2.1.5 Geographical Distribution of Non-Bitter *Cucumis metuliferus*

Non-bitter *Cucumis metuliferus* has existed as a wild plant throughout the tropical and subtropical sub-Saharan regions of Africa. It has also been found in countries in the Middle East like Yemen, Croatia Europe, and Australia. The plant is found in many parts of Kenya, including the eastern, western, and central regions (Owino *et al*., 2020). In Kenya, New Zealand, France, and Israel, the fruits are commercially grown for export (Wannang *et al*., 2008). The plant grows well in well-drained sandy or loamy soil, although it has been found in clay soils and sloppy rocks. The plant has been collected at altitudes ranging from 210 to 1800 metres above sea level. It does well in warm climates, although it can adapt to a quite wide range of temperatures (Mey, 2016).

2.1.6 Phytochemical Composition of Non-Bitter *Cucumis metuliferus* Plant

Phytochemical constituents of the non-bitter CMFE depend on soil types, treatment conditions, the part of the plant used in the extraction and other geographical factors (Maluleke, 2022). According to Dhale (2011), the non-bitter *Cucumis metuliferus* fruit pulp contains glycosides, alkaloids, saponins, and tannins. Šeregelj *et al*. (2022) established that *the Cucumis metuliferus* plant has flavonoids, phenols, and beta-carotene in the fruit pulp; flavonoids, phenols, tannins, alkaloids, steroids, glycosides, and saponins in the rind; and flavonoids, phenols, tannins, alkaloids, steroids, beta-carotene, saponins, fatty acids and tocopherols in the seed. Ezekaibeya *et al. (2020)* also demonstrated that *Cucumis metuliferus* rind contains glycosides, alkaloids, flavonoids, saponins, steroids, tannins, terpenoids, and phenols.

2.1.7 Acute Oral Toxicity Profile of the Non-Bitter *Cucumis metuliferus* Fruit Extract

The toxicological potential of herbal medicine products is affected by environmental and plant factors and to ensure the safety of consumers, a toxicological assessment must be conducted before use (Jităreanu *et al*., 2023). In a study by Wannang *et al*., (2008), an oral dose of 10, 100, 1000, 1500, 3000, and 5000mg/kg body weight, the non-bitter CMFE produced no lethal toxicity to Wistar albino rats. Similarly, Usman *et al.*, (2018), demonstrated that at an oral dose of 500mg/kg body weight, the non-bitter CMFE did not produce signs or symptoms of toxicity in cockerels.

2.2 Diabetes Mellitus

2.2.1 Definition and classification of Diabetes Mellitus

Diabetes is an endocrine metabolic disorder hallmarked by persistent hyperglycemia due to a lack of insulin secretion, insulin action, or both. The two most common forms of diabetes are type 1 diabetes and type 2 diabetes both of which are caused by a combination of genetic and environmental risk factors (Khawandanah, 2019).

Type 1 diabetes mellitus, formerly named insulin-dependent diabetes mellitus, is caused by insulin deficiency following auto-immune destruction of the pancreatic beta cells. Despite extensive investigations into the pathogenesis of type 1 diabetes, the underlying causes and mechanisms are poorly understood. It is one of the most common chronic diseases in childhood, and lifelong supplementation of insulin or its analogues is their only hope for survival (Roep *et al.* 2021).

Type 2 diabetes mellitus is caused by a combination of impaired insulin secretion and insulin resistance in the peripheral target tissues, especially muscle and the liver. This type of diabetes is most often associated with older age, obesity, a history of diabetes in the family, a previous history of gestational diabetes, physical inactivity, and certain ethnicities. It accounts for about 90% of all cases of diabetes mellitus (Galicia *et al*., 2020).

2.2.2 Economic Burden of Diabetes Mellitus:

Globally, it is estimated that the cost of management of diabetes mellitus in 2030 will have risen by 61%, which will translate to more than 2.1 trillion USD (Bommer et al., 2018). In sub-Saharan Africa, the most affected age group is in the bracket of 20 to 44 and 45 to 64, which is the most economically productive (Mercer et al., 2019). In Kenya, about half a million patients with diabetes mellitus reported in 2019 used about 229 million USD to cater for treatment, whereas about 144 million USD was used indirectly (Adamjee & Harerimana, 2022). Oyando et al. (2020), reported that about 29.5% of patients stopped working because of diabetes mellitus illness, while 33% lost about 21 working days in 90 days due to diabetes. According to Masis et al. (2022), a Kenyan type 2 DM patient spends about 488.60 USD to access medical services in a private hospital and about 88.61 USD in a public hospital

2.2.3 Metabolic Disorders in Diabetes Mellitus

Dyslipidaemia: According to Kumar *et al.* (2022), poor glycaemic control over a prolonged period in diabetes mellitus is inversely proportional to levels of HDL. It was observed that an increase in Hb A_{1c} (the test used to assess long-term blood sugar control) led to a decrease in HDL with a P-value of 0.02. In diabetes mellitus, due to insulin resistance or deficiency, there is low cellular glucose uptake, which in turn signals mechanisms responsible for overt hyperglycemia. One of the mechanisms is lipolysis, which is responsible for liberating fatty acids from adipocytes and making them available to the liver to be used in gluconeogenesis. This eventually leads to an imbalance between HDL, LDL, and VLDL, where HDL decreases and the latter increases, predisposing the patient to atherosclerotic cardiovascular diseases (Schofield *et al*., 2016; Dixit *et al.* 2014; Ginsberg, 1996) stated that hypercholesterolemia, hypertriglyceridemia, and elevated LDL are the lipid abnormalities associated with diabetic dyslipidemia.

Liver cell disorder: Insulin resistance in type 2 DM may be aggravated by oxidative stress and aberrant inflammatory signals, becoming one of the main contributing factors

to liver damage (Mohamed *et al.,* 2016a). Hepatocellular damage characterized by raised AST, ALT, and ALP enzymes in diabetes mellitus results, and this could be due to the direct hepatotoxic effect of fatty acids on the liver produced in excess following hepatic cells insulin resistance. Mechanisms involved may include cell membrane disruption at high concentrations, mitochondrial dysfunction, toxin formation, and activation and inhibition of key steps in the regulation of metabolism (Mathur *et al.*, 2016).

Effects of diabetes mellitus on body weight: Gluconeogenesis, induced by the failure of cells to take up glucose due to insulin resistance or deficiency, depletes the stored amino acids and fatty acids. The process is augmented by the induction of glycogenolysis, and the overall result is overt hyperglycemia. This, together with chronic diabetic complications like kidney failure and heart failure, has been associated with unintentional weight loss and an increased mortality rate in diabetic patients (Xavier, 2018).

2.3 Experimental Induction of Diabetes Mellitus

Induction of diabetes in animal models can be done by introducing chemicals into the body that target and destroy the beta cells of the pancreas, surgical removal of the pancreas, or genetic engineering of diabetes in an animal (Etuk, 2010). According to Al-awar *et al*. (2016), diabetic rat models play an important role in elucidating the pathogenesis of human diabetes and related complications, in this case, rats have been widely used for investigating and developing novel drugs for the disease and its complications.

2.3.1 Chemical Induction of Diabetes Mellitus Using Streptozotocin

Etuk (2010) stated that the majority of studies employ chemical induction of diabetes mellitus in animal models, and streptozotocin is by far one of the most commonly used drugs. The drug is administered through the parenteral route, but the dose required for inducing diabetes depends on the animal species, route of administration, and nutritional status (Graham *et al.,* 2011). When administered, streptozotocin induces insulin

deficiency by selectively diffusing through the pancreatic beta cell membrane with the help of the carrier protein glucose transporter 2 (GLUT2), which eventually leads to the death of the beta cells via necrosis (Eleazu *et al*., 2013). However, streptozotocin is considered the agent of choice for inducing diabetes mellitus in experimental animals (Lenzen, 2008). When administered in a high-fat diet fed to rodents, low-dose streptozotocin produces type 2 diabetes (Furman, 2021).

2.4 Summary

Jimam *et al*. (2010) and Gotep (2011) described the possibility of using *Cucumis metuliferus* fruit in the treatment of Diabetes. However, studies reviewed suggest that further investigation of the potentials of *C. Metuliferus* in the management of diabetes mellitus be done to pave the way for drug development. Other studies recommend further investigation of *C. metuliferus* fruit to fully establish its safety profile (Jimam *et al*., 2012; Usman *et al*., 2018). This present study sought to tackle some of the objectives not tackled by the studies reviewed

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The study was conducted at the Small Animal Facility for Research and Innovation (SAFARI) at Jomo Kenyatta University of Agriculture and Technology (JKUAT), Kenya, between December 2019 to April 2020. SAFARI is located next to the College of Health Sciences building. The facility is under the management of JKUAT and is meant for research and innovation. Animals are kept in appropriate animal cages and handled by trained animal technicians. The sacrifice of experimental rats and collection of specimens was conducted in SAFARI's procedure room. Collected blood specimens were processed and analysed in the biochemistry laboratory, while tissue specimen processing and analysis were done in the histology laboratory at JKUAT.

3.2 Study Design

A laboratory-based experimental study design was adopted.

3.3 Type of Animal Model Used

According to Sood e*t al.* (2013), diabetes mellitus disease progression in the Wistar rat's model is comparable to human diabetes mellitus. In this study, Wistar albino rats were used.

3.3.1 Acquisition of Wistar Albino rats

All the rats were acquired from the JKUAT SAFARI animal house, where breeding was done up to the 5^{th} generation to obtain a pure colony of Wistar Albino rats.

3.3.2 Selection of Wistar AlbRatsrats

Five (5) week-old Male Wistar albino rats weighing between 90 and 130 grammes were selected and included in this study.

3.3.3 Wistar Albino Rats handling

The rats were handled humanely, and the rules and regulations of SAFARI Animal House were adhered to at all times. They were kept in polypropylene rat cages measuring 410 x 285 x 180 mm per cage. The rats were subjected to a 12-hour light/dark cycle and had free access to approved rodent pellet food and clean water according to the experimental protocol.

3.4 Sample Size Calculation

The sample size was arrived at using the "resource equation method" (Charan and Biswas, 2013).

E= Total number of animals – Total number of groups

Total number of animals = No. of animals per group x No. of groups

E = (No. of groups x No. of animals per group) – No. of groups

E is the degree of freedom of ANOVA and its value is considered scientifically adequate if it lies between 10 and 20

In this study;

Number of groups = 4

Number of animals per group = 4

Total number of animals =4 x 4

E= 16-4=12

Since the E (12) value was between 10 and 20, the data collected in this study was considered scientifically adequate.

However, the number of animals in each group was multiplied by 4 to cater for the interval of sacrifice on days 45, 56, 63, and 70 of the experiment. This gave 16 rats in each group and a total of 64 rats.

3.5 Grouping of the Rats

The sixty-four (64) male Wistar Albino rats were weighed and randomly assigned into two major groups, i.e., the control group and the experimental group. The experimental group received a high-fat and fructose diet + streptozotocin (STZ) injection to induce diabetes mellitus, whereas the control group received a standard rodent pellet diet + 0.9% normal saline as a placebo. The experimental group was further divided into treatment group 1 (the positive control group) which was treated with Pioglitazone at a dose of 20mg/kg body weight, treatment group 2 which was treated with low-dose CMFE at 200mg/kg body weight, and treatment group 3 that was treated with high-dose CMFE at 400mg/kg body weight. All the groups had sub-groups "a", "b", c," and "d" each to cater for the serial sacrifice on the 45^{th}, 56^{th}, 63rd, and 70th days of the experiment.

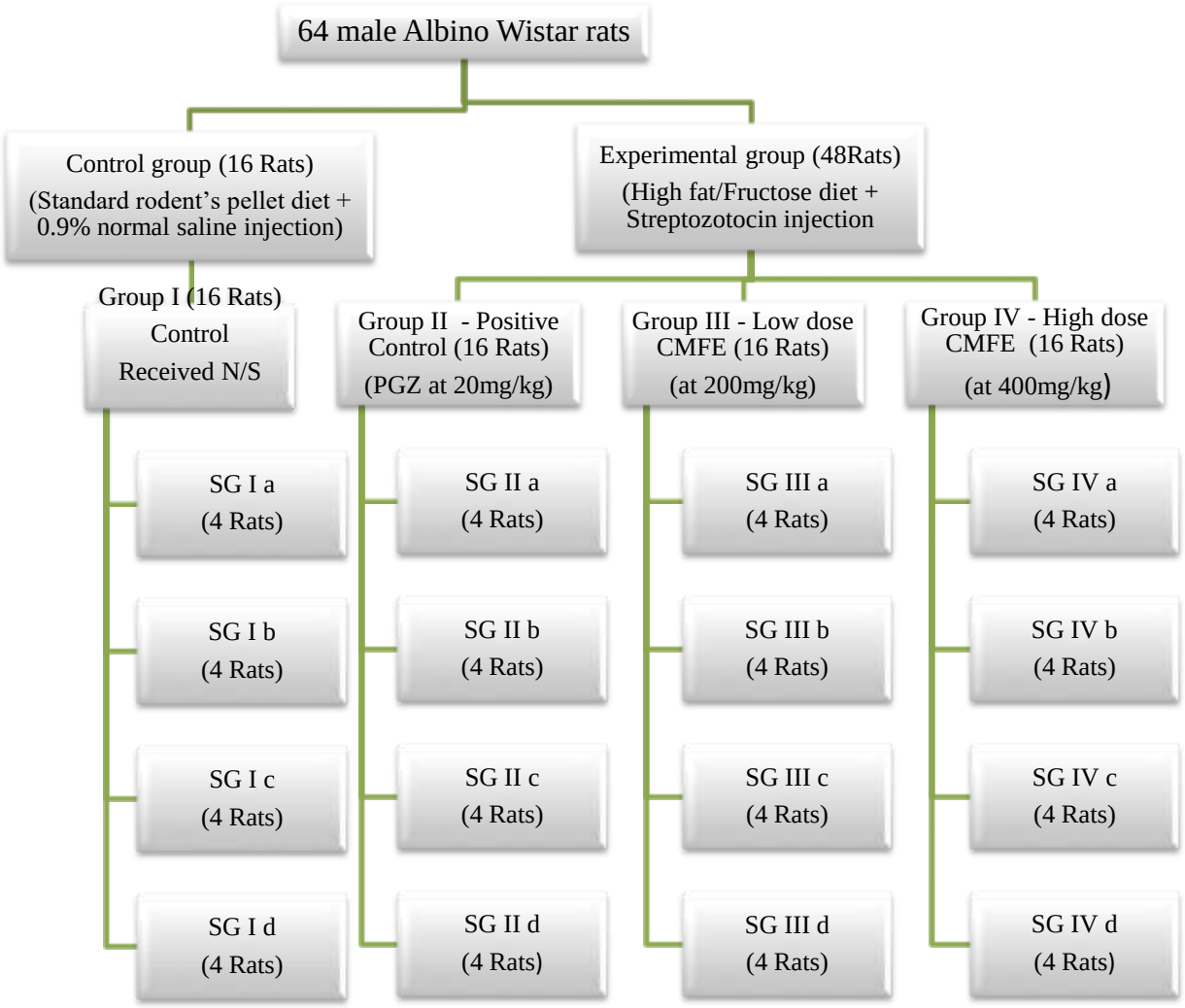

Figure 3.1: Grouping of Animals and Treatment

3.6 Harvesting of Non-Bitter *Cucumis metuliferus* Fruit

The non-bitter form of *Cucumis metuliferus* fruits was harvested from a farm in Wamumu ward, Mwea West Sub-County, Kirinyaga County, with the help of a plant taxonomist and later taken to the Department of Botany at Jomo Kenyatta University of Agriculture and Technology for identification and confirmation, and a voucher specimen (Voucher No. DMM-JKUATBH 001A-2019) was deposited in the JKUAT botany herbarium for future reference. Wamumu ward has an altitude of 1158 meters and lies along 37.373^0 E and 0.738^0 S. The area receives an average annual rainfall of between 1200 and 1600mm, while temperatures range between 14.37^o C and 32.17^o C annually. The area is covered with black cotton soil (Mwai et al., 2021).

3.6.1 Extraction of Non-Bitter *Cucumis metuliferus* Crude Fruit Extracts

Extraction of the fruit was done using the maceration technique as described by Zhang *et al*. (2018). Whole fruits were thoroughly washed of dirt and any foreign bodies using clean water, and then air dried. The fruits were then cut into two halves; the contents were scooped using a spatula, put in a collection jar, and then soaked in ethanol in an airtight glass vessel. At room temperature, the mixture was allowed to stand while occasionally shaking in an orbital shaker for 48 hours. The liquid was then strained off and the solid residue was pressed to recover as much of the occluded solution as possible. The solution was then filtered to remove particles using a muslin cloth, followed by filter paper. Ethanol was then evaporated using a rotary evaporator, and freeze drying followed to retain *Cucumis metuliferus* fruit extract paste. The paste was stored at 4 degrees Celsius until use.

3.7 Preparation of a High-Fat and Fructose Diet for the Rats

In the preparation of a high-fat diet, the study adopted a protocol developed by Liu *et al.* (2017) which states that if more than 35% of calories in a diet come from fat, the food is considered to be a high-fat diet. A high-fat diet was prepared by mixing vegetable cooking

fat (‘Frymate) manufactured by Pwani Oil Products, Kenya, with standard rat pellets from Unga Feeds Limited, which had the following composition: carbohydrates (65.3%), crude protein (18.1%), crude fibre (7%), fat (8%), calcium (0.8%), and phosphorous (0.8%). 15 grams of Frymate (which provided 900 Kcal per 100-gramme serving) were mixed with 100 grammes of standard rat pellets, and the mixture was allowed to heat for 15 minutes in a cooking pan placed over an electric cooker set at 50 degrees Celsius. During the heating process, the mixture was continuously stirred for proper mixing and fat penetration into the pellets. The end product had the following composition: carbohydrates (55.5%), fat (21.8%), crude protein (15.4%), crude fibre (6%), calcium (0.7%), and phosphorous (0.7%), making 37% of the calories come from fat. In preparation for a fructose diet, this study adopted a protocol developed by Wilson & Islam (2012), which states that rats subjected to 10% fructose solution *ad libitum* before and after treatment with low-dose streptozotocin injection to develop type 2 diabetes had higher chances of survival over eight weeks post-injection than rats fed on a higher fructose amount. A fructose solution was prepared by pouring distilled water into a jar containing 10 grammes of fructose while stirring to make a 100-mL solution. The experimental rats had access to the fructose solution *ad libitum* throughout the experiment.

3.8 Feeding of the Rats

The control group was fed standard rat pellets containing carbohydrates (65.3%), fat (8%), crude protein (18.1%), crude fibre (7%), calcium (0.8%), and phosphorous (0.8%), while the experimental group was fed a high-fat diet containing carbohydrates (55.5%), fat (21.8%), crude protein (15.4%), crude fibre (6%), calcium (0.7%), and phosphorous (0.7%) plus Fructose solution. All rats received food and water *ad libitum.*

3.9 Phytochemical Screening

The phytochemical screening was done using standard phytochemical testing procedures as described by Gul *et al*. (2017).

Test for Glycosides

1 ml of extract was mixed with 1 ml of glacial acetic acid and then treated with one drop of a 5% ethanolic chloride solution. 1 ml of concentrated sulfuric acid was carefully poured down the side of the test tube. The appearance of a brownish ring between the two formed layers, with the lower acidic layer turning blue-green upon standing, would indicate the presence of cardiac glycosides.

Alkaloids Test

1 ml of the extract was tested with Mayer's reagent, prepared by dissolving 35g of mercury chloride in distilled water and a solution of 5 grams of potassium iodide in 10 ml of water. The mixture was diluted to 100 ml. The appearance of opalescence or a yellow precipitate would indicate the presence of alkaloids.

Flavonoids Test

1 ml of the extract was put into a test tube, followed by the addition of hydrochloric acid (4 drops) and magnesium turnings. The development of a pink or magenta red would indicate the presence of flavonoids.

Sterols and Steroid Tests

1 ml of the extract was put into a test tube to which 0.5 ml of sulfuric acid, acetic anhydride, and chloroform in similar amounts were added. A red colour would indicate the presence of sterols. A green colour would indicate the presence of steroids.

Saponins Test

1 ml of the extract was put in a test tube, and then 50 ml of tap water was added. The mixture was shaken vigorously, and if persisting honeycombs formed, these would be subjected to confirmatory tests. This involved dissolving 1 ml of the extract in anhydride

tetrachloride, to which 4 drops of concentrated sulfuric acid were added. A blue, green, or red colour accompanied by a pink ring would show the presence of saponins.

Tannins Test

1 ml of the extract was dissolved in water, to which 1% gelatin salt reagent containing 1% gelatin and 10% sodium chloride and a salt solution (10% NaCl) was added. The presence of tannins would be indicated by the presence of a blackish-blue colour, while catechol tannins would be a greenish-black colour.

3.10 Acute Oral Toxicity Study of Non-Bitter *Cucumis metuliferus* Fruit extract

An acute oral toxicity study was conducted using the up-and-down procedure as described by the Organisation for Economic Co-operation and Development (2001) test guideline number 423.

Twelve (12) nulliparous and non-pregnant Wistar Albino rats weighing between 170 and 190 grammes and aged 8 weeks were procured and kept in appropriate rat cages in the JKUAT SAFARI animal house. The rats were treated to a 12-hour light/dark cycle and had free access to approved rodent pellet food from Unga Feeds Limited and clean water. They were handled humanely, and the rules and regulations of SAFARI Animal House were adhered to at all times.

3.10.1 Testing Procedure

Twelve rats were divided into four groups of three rats each. Group 1 served as the control and was given distilled water. Groups 2, 3, and 4 were treatment groups and were given CMFE at doses of 50mg/kg, 300 mg/kg, and 2000 mg/kg, respectively. The starting dose was selected from one of the four fixed levels: 5, 50, 300, or 2000 mg/kg, according to OECD test guideline number 423. Before dosing, rats were fasted overnight for 6–8 hours, weighed, and, after dosing, food was withheld for 4 hours. However, they had free access to clean water during the fasting period. The calculated dose of freeze-dried CMFE was

formulated into an aqueous solution by adding distilled water to make a concentration of 1 ml: 200 mg CMFE. Dosing was done at intervals of 48 hours from one step to the next.

Observation for signs and symptoms of toxicity was made by paying special attention to skin and fur changes, eye and mucous membrane changes, and weight changes. The rats were also observed for diarrhoea, salivation, tremors, convulsions, lethargy, sleep, coma, and death.

3.11 Confirmation of Non-Diabetic status before Treatment with Streptozotocin

Before induction of type II DM, the rats fasted for 6–8 hours during the early morning hours. A drop of whole blood was collected through a tail prick to test for fasting blood sugar (FBS), followed by a 2-hour oral glucose tolerance test (OGTT), and the readings were recorded to confirm that all the rats were non-diabetic before induction i.e., the rats had a FBS level of less than 7 mmol/L with an average OGTT of less than 11.1 mmol/L (Patel & Macerollo, 2010). FBS and OGTT were tested using the "Accu Chek" glucometer manufactured by Roche Diabetes Care Company in Indiana, USA. Using an electronic weighing machine, the rats were weighed and their body weights were recorded.

3.12 Induction of Diabetes Mellitus Using Streptozotocin

Streptozotocin at a dose of 35 to 65 mg/kg body weight administered intraperitoneally or intravenously induces diabetes mellitus in rats (Islam et al., 2017). Etuk (2010) stated that a single intraperitoneal injection of streptozotocin at 60mg/kg in rats produces type 2 diabetes mellitus.

In this study, streptozotocin (STZ) was administered through intra-peritoneal injection using the standard operating procedures for intra-peritoneal injection of drugs into rats as described by Andrews (2014).

On day 42 of the experiment, the rats fasted for 6–8 hours in the early morning hours before being administered with a single intraperitoneal STZ injection. At 40 mg/kg body

weight, streptozotocin powder was dissolved in 0.9% normal saline to form a solution. The freshly prepared solution was injected through the intraperitoneal route into all the rats in the experimental group. The control group received a single intraperitoneal injection of 0.9% normal saline. Food was withheld for four hours following treatment.

3.13 Reconstitution of Streptozotocin (STZ) Chemical

Streptozotocin was reconstituted according to the instructions provided by the manufacturer (MedChemExpress, USA), where 0.9% normal saline was used. After reconstitution, the drug was administered immediately to avoid degradation.

3.14 Confirmation of Diabetes Mellitus (DM)

After six (6) hours of fasting, fasting blood sugar (FBS) was tested, followed by a 2-hour oral glucose tolerance test (OGTT). Glucose was administered via oral gavage at a dosage of 2g/kg body weight. Blood sugar levels were tested at 30 minutes, 1 hour, 1.5 hours, and 2 hours post-oral glucose. The rats that had a fasting blood glucose level of 7 mmol/L or above with an average OGTT of more than 11.1 mmol/L were considered diabetic (Patel & Macerollo, 2010). After confirmation of the diabetic state, the treatment commenced.

3.15 Determination of Dosage of *Cucumis metuliferus* Fruit Extract

According to Jimam et al. (2011), a dose of 1000mg/kg of *Cucumis metuliferus* fruit extract led to tissue necrosis and degeneration of the liver, while the kidneys showed features of renal epithelial cell damage. However, a dosage of 500mg/kg body weight had no significant alteration of liver or renal tissues. The tissues of the spleen and pancreas of rats treated with both doses of 500mg/kg and 1000mg/kg of *Cucumis metuliferus* fruit extract were normal when compared with the control group. A study by Gotep (2011) showed that diabetic rats treated with 100mg/kg of CMFE had blood sugar levels comparable to those of those treated with chlorpropamide within 24 hours. Since there was no human data that could be used to calculate the dosage of *Cucumis metuliferus* crude extract, based on the acute oral toxicity study of CMFE conducted before the current

study and the above references, a dose of 400mg/kg body weight was adopted as the maximum therapeutic dose (high dose) and 200mg/kg body weight as the minimum therapeutic dose (low dose) of CMFE. The low dose was calculated using the natural logarithm function from the highest dose of the crude extract. In this study, the desired potency of the crude extract was 90%.

Least dose=Highest dose x e^ of 90%

Highest dose=400

e^ of 90%=1-0.9=0.1=0.1054

Least dose=400 x e^ (-0.1054) =Approx. 200

3.16 Preparation and Administration of *Cucumis metuliferus* Fruit Extract Solution

CMFE paste was reconstituted with 5% DMSO to make a solution containing 100mg/ml CMFE. The extract was administered at doses of 200mg/kg (low dose) and 400mg/kg (high dose) via oral gavage.

Treatment commenced immediately after confirmation of diabetes mellitus on day 45 and continued until day 70 when the experiment was terminated.

3.17 Pioglitazone Solution Preparation, Calculation, and Administration

This study adopted a dose of 20mg/kg of pioglitazone as described by Chege *et al.* (2019). For easy administration, a drug solution was prepared by dissolving the 30mg PGZ tablet in 5% DMSO to make a concentration of 5mg/ml of PGZ. The drug was administered via oral gavage for 28 days.

3.18 Humane Killing of the Rats

This activity was carried out in the SAFARI procedure room. The rats were fasted for 6–8 hours before being sacrificed.

3.18.1 Procedure for Anaesthetizing The Rats and Collecting the Specimens

The rat was put into a bell jar containing cotton wool, and the jar was covered. While still covered and via plastic tubing connected to a regulator attached to the gas cylinder, 70% concentrated carbon dioxide was introduced for 1 minute. After 3-5 minutes, anaesthesia took effect, and the anaesthetized rat was removed from the bell jar and mounted onto the board using mounting pins with the dorsal side on the board. Using a pair of scissors and forceps, the rat was cut through the ventral medial side from the symphysis pubis to the sternal angle of the thoracic cage, and both intra-thoracic and abdominal structures were exposed for easy access.

Using a 5 cc syringe with a hypodermic needle, approximately 5 ml of blood was collected from the heart through direct intra-cardiac puncture and immediately emptied into the appropriately labelled collecting test tubes. The samples were stored in a refrigerator at temperatures between 2 and 8 degrees Celsius before being packed in a cold box and transported to the biochemistry laboratory for biochemical tests.

A perfusion needle connected to the perfusion set was then inserted into the left ventricle of the heart, and the remaining blood was cleared by running 200 ml of 0.9% normal saline by force of gravity from the drip set. After sufficient clearing, the saline drip was removed, and using the same perfusion needle, 10% formalin solution, a fixative, was introduced slowly into the circulation of the rat. The firmness of the tail was checked as a sign of effective fixation.

The pancreas was then carefully excised and immersed in a clearly labelled container with fresh fixative to continue fixation for at least 12 hours. Later, the samples were then transported to the Histology laboratory for histo-morphological analysis.

3.19 Body Weight Measurement

Rats were weighed and their body weights were recorded on days 1, 7, 14, 21, 28, 35, 42, 49, 56, 63, and 70 of the experiment.

3.20 Biochemical Tests

Blood tests were carried out in the biochemistry laboratory at JKUAT.

3.20.1 Testing of Haemoglobin A1c

Hb A1c was tested on days 45, 56, 63, and 70 of the experiment using the Clover A_{1c} Self Hb A_{1c} Analyzer manufactured by Infopia Co., Ltd.

About 4 microliters of whole blood were collected using a capillary pipette and put on the collection leg of the reagent pack.

The reagent pack was then inserted into the cartridge, where the blood was instantly lysed, releasing the haemoglobin. The cartridge was then inserted into the clover A1c analyzer, and the blood sample mixture was rotated to the measurement zone of the cartridge, where the amount of total Hb in the blood was measured by the reflectance of the photo sensor light-emitting diode and photodiode. The assembled cartridge was then rotated so that the washing solution could wash out non-glycated Hb from the blood sample so that glycated Hb could be photometrically measured. The ratio of glycated Hb with respect to total Hb in the blood sample was then calculated by the machine, and the readings were displayed on the screen. The readings were then recorded in the logbook, and both the lancet and the spent cartridge were discarded appropriately. The results were expressed in percentage, with normal HbA1c ranging between 4% and 6.4% (Patel & Macerollo, 2010).

3.20.2 Testing of Fasting Blood Sugar and Oral Glucose Tolerance (Pickering & Marsden, 2014)

Fasting blood sugar (FBS) and oral glucose tolerance test (OGTT) were tested on days 42, 45, 56, 63, and 70 of the experiment, and the tests were carried out using an "ACCU CHEK" glucometer (Roche Diabetes Care Inc.).

After fasting the rats for 6–8 hours, each rat was put in a rodent Restrainer. When it was safely secured, its tail was cleaned using an alcohol-benzocaine pad (Benzocaine 6%, isopropyl alcohol 70% v/v) and allowed to air dry for approximately 30 seconds. One test strip was then inserted into the test strip slot of the glucometer, and the glucometer turned on automatically. A code number matching the one on the test strip package automatically appeared on the screen. When the blood drop symbol flashed on the glucometer, using a lancet, the rat was pricked on the lateral tail vein, and a drop of whole blood was gently squeezed out. The glucometer was then picked up, and the edge of the application point of the test strip was brought close enough to touch the blood drop, and the blood was drawn into the test strip automatically. A beep sound from the glucometer was heard as an indication that the testing had begun, followed by the display of the readings on the screen. The readings for FBS were then recorded on the logbook, and the test strip was removed from the glucometer and discarded in a puncture-proof container. The lancet was also discarded in a sharp box. After testing for FBS, glucose was then administered via oral gavage at a dose of 2g/kg body weight, and using the same procedure as for testing FBS, OGTT was tested at 30 min, 60 min, 90 min, and 120 min. The average of the four readings was calculated and recorded in the logbook.

The rats that had a fasting blood glucose level of 7 mmol/l or more plus an average OGTT of/ more than 11.1 mmol/l were considered diabetic (Patel & Macerollo, 2010).

3.20.3 Testing of Liver Functions (Kanagasabapathy & Kumari, 2000)

Rats were fasted for 6–8 hours before collecting the blood specimen. At least 4 ml of whole blood was collected through intra-cardiac puncture, put in a plain test tube, and transported to a biochemistry laboratory for processing. Blood was allowed to clot for 15 to 20 minutes, followed by centrifugation to obtain serum. The CS T240 auto chemistry analyzer machine (Dirui Industrial Company Limited) was used to test for serum Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Gamma-glutamyltransferase (GGT), Triglyceride, Total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), Albumin, total protein, total bilirubin, and direct bilirubin. Testing was done according to the laboratory procedure manual provided by Dirui Industrial Company Limited.

ALT	5 to 40 U/L
AST	8 to 34 U/L
ALP	40 to 150 U/L
GGT	Less or equal to 50 U/L
Triglycerides	0.45 to 2.3 mmol/L
Total cholesterol	3.1 to 6.1 mmol/L
LDL	Less or equal to 3.1 mmol/L
HDL	1.07 to 6.0 mmol/L
Albumin	37 to 53g/L
Total Protein	60 to 83g/L
Total Bilirubin	3.4 to 17.1 micromole/L
Direct bilirubin	1.7 to 6.8 micromole/L

3.21 Histo-morphological studies

Histo-morphological studies were carried out in the histology laboratory at Jomo Kenyatta University of Agriculture and Technology. The rats were fasted for 6–8 hours before being sacrificed. The pancreas was harvested from both the control and experimental group on days 45, 56, 63, and 70 according to the experimental protocol.

3.21.1 Processing the Pancreas for Light Microscopy (Kahyaolu & Gökçimen, 2017)

Preserved tissues were dehydrated in an ascending concentration of absolute alcohol (50%, 70%, 80%, 90%, 95%, and 100%) for an hour in each grade, then cleared in toluene for 20 minutes. The tissues were then infiltrated with paraplast wax for 12 hours at 56 degrees Celsius. The pancreatic tissues were then oriented along the longitudinal axis and embedded in tissue cassettes, ready for microtomy. Using a Leitz sledge rotary microtome, tissues were then sectioned at five (5) micrometre thickness. The sections were then floated in a 37-degree water bath for a minute to spread the tissue. Using a glass slide smeared with egg albumin, the sectioned tissues were fished out, followed by drying the slides in an oven at 37 degrees for 24 hours in readiness for staining.

3.21.2 Method for Staining

Pancreatic tissue sections were stained using haematoxylin and eosin as described by Cardiff *et al*. (2014). Excess wax on the tissues was removed by soaking them in xylene for 4 minutes. This was followed by the removal of excess water from the sections by introducing them in descending grades of alcohol, i.e., 100%, 95%, 80%, 70%, and 50%, for three (3) minutes in each grade. The tissues were then stained with Harris hematoxylin for 10 minutes and then subjected to running tap water for 30 minutes. Staining with eosin for 7 minutes followed, and the sections were then dehydrated by introducing them to ascending graded alcohol, i.e., 50%, 70%, 80%, 90%, and 100%, for 3 minutes in each grade.

Prepared slides were cleaned in xylene and then covered using DPX coverslips in readiness for microscopy, which was done using a light microscope under power x40, x100, x400, and x100.

Photomicrographs were acquired by using a light microscope connected to a SWIFTCAM SC2003 camera manufactured by Koto-Ku, Tokyo, Japan. The images were acquired and stored on a computer in the joint photography expert group (JPEG) image format. After

analysis and labelling of the photomicrographs, they were then saved in tagged image format (TIF).

3.22 Data Analysis

Quantitative data for FBS, OGTT, HbA1c, total cholesterol, triglycerides, LDL, HDL, AST, ALT, GGT, ALP, total bilirubin, direct bilirubin, total protein, albumin and body weight of the rats was keyed into a Microsoft Excel spreadsheet and later transferred to Statistical Package for Social Sciences (SPSS) version 25. Means were calculated and ANOVA was used to compare multiple means. The Tukey statistical test was used for post-hoc statistical analysis. The analysis was done at a 95% level of confidence ($P < 0.05$). Qualitative data like the change of colour in the phytochemical screening of the non-bitter CMFE, clinical features of the rats in the acute oral toxicity test, and histomorphology results of the pancreatic tissues were reported through the description and labelling of photomicrographs. Tables and figures were used to present the data. Results were disseminated to the university and research community as thesis, department seminars, and publications in peer-reviewed journals.

3.23 Ethical Consideration

Ethical approval was sought from the JKUAT Animal Ethics Review Committee. Ethical conduct was observed at all times during the entire period of the study. Animals were not used for any other purpose other than that indicated in the proposal.

CHAPTER FOUR

RESULTS

4.1 Non-Bitter *Cucumis metuliferus* fruit Extract Yield

The ripe fruit had a percentage yield of 1.15 following ethanol extraction through the maceration technique (Table 4.1).

Table 4.1: Percentage yield of Non-Bitter *Cucumis metuliferus* fruit extract

Plant	Weight of the fruit extract (Raw)	Weight of freeze-dried extract (Paste)	Percentage yield
Cucumis metuliferus fruit (Ripe)	3200 grams	36.7 grams	1.15

4.2 Phytochemicals Present in Non-Bitter *Cucumis metuliferus* Fruit Extract

Phytochemical screening revealed that *Cucumis metuliferus* ethanol fruit extract glycosides, alkaloids, saponins, and Tannins. Flavonoids, sterols, and steroids were absent (Table 4.2).

Table 4.2: Phytochemicals Present in Non-Bitter *Cucumis metuliferus* Ethanol Fruit Extract

Phytochemical	Quantity
Glycosides	+
Alkaloids	+
Flavonoids	-
Sterols and Steroids	-
Saponins	+
Tannins	+

Key

"+" means present

"-"means absent

4.3 Acute Oral Toxicity Study of *Cucumis metuliferus* Fruit Extract

Dosing at 50mg/kg and 300mg/kg produced no signs or symptoms of acute Oral toxicity. However, there was increased physical activity and a change in behaviour patterns (jumping, movement within the cage, making noise, and eating habits) at a dose of 2000mg/kg CMFE. In all the treatment groups, there were no changes observed for skin and fur, eyes, or mucous membranes. Moreover, the rats did not present with diarrhoea, salivation, tremors, convulsions, lethargy, drowsiness, or coma as well. There was no mortality recorded in this study (Table 4.3a).

There was no significant statistical difference (P = 0.723) when mean body weights were compared between the control group and the group treated with CMFE at various toxicity test doses, suggesting CMFE was not toxic and hence there was no change in body weight (Table 4.3b).

Table 4.3a: Acute Oral Toxicity Observation List Results for *Cucumis metuliferus* Fruit Extract

Group	Treatment	Route of administration	Observations schedule						Mortality
			0 Hr	½ Hr	1 Hr	24Hrs	48Hrs	Daily for 14 days	
1	Distilled water	Oral gavage	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	0/3
2	CMFE 50(Mg/Kg)	Oral gavage	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	0/3
3	CMFE 300(Mg/Kg)	Oral gavage	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	0/3
4	CMFE 2,000(Mg/Kg)	Oral gavage	Normal activity	Normal activity	Increased physical activity and eating habits	Increased physical activity and eating habits	Increased physical activity and eating habits	Normal activity	0/3

Table 4.3b: Comparison of Mean Body Weight between Various Toxicity Test Groups

Groups	N	Mean	Std. Deviation
Group 1 (Control)	12	202.37	19.15883
Group 2 (50mg/kg CMFE)	12	207.53	16.96758
Group 3 (300mg/Kg CMFE)	12	204.31	17.83186
Group 4 (2000mg/Kg CMFE)	12	199.18	18.72542

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	440.961	3	146.987	.444	.723
Within Groups	14559.339	44	330.894		
Total	**15000.300**	**47**			

4.4 Effects of *Cucumis metuliferus* Fruit Extract on HbA1c

There was no significant statistical difference (P =0.224) in Hb A_{1c} between the control group and the treatment groups on day 45 of the experiment following induction of type II DM, suggesting that induction of type II DM did not have an immediate effect on HbA1c. However, there was a significant statistical rise (P =0.006) in Hb A_{1c} between the control group and the treatment groups on day 56 of the experiment, with no significant statistical difference on days 63 (P =0.079) and 70 (P =0.712) of the experiment. Post-hoc statistical analysis using the Tukey test revealed a significant statistical rise (P = 0.006) of Hb A_{1c} in the positive control group (6.73±0.945 test vs 4.767±0.153 control) on day 56 of the experiment (Table 4.5). These results demonstrate that CMFE chronically controlled the blood sugar, as evidenced by sustained mean Hb A_{1c} to levels comparable to the control group and less than 6.5% throughout the experiment. However, a significant rise in Hb A1c on day 56 of the experiment in the positive control group demonstrates that the non-bitter CMFE achieved control of blood sugar earlier than the pioglitazone (Table 4.4).

Table 4.4: Comparison of Mean Haemoglobin A1c between Various Treatment Groups

Test Day	Control	Positive Control –PGZ (20mg/kg)	Low dose CMFE 200mg/kg	High dose CMFE (400mg/kg)	F	*p*-value
45 days	4.700±0.200	1.644±2.468	1.600±2.402	1.622±2.436	1.557	0.224
56th day	4.767±0.153	6.73±0.945*	5.033±0.115	5.233±0.351	8.864	0.006
63rd day	4.867±0.208	4.933±0.153	4.833±0.153	5.500±0.520	3.284	0.079
70th day	5.067±0.231	5.033±0.153	4.97±0.153	5.133±0.153	0.468	0.712

Tukey test on post-hoc (mean difference)

Test Day	Control	Positive Control – PGZ (20mg/kg)	Low dose CMFE 200mg/kg	High dose CMFE (400mg/kg)
45 days	0	3.06(-1.23 – 7.34)	3.10 (-1.18 – 7.38)	3.08(-1.20 – 7.36)
56th day	0	-1.97(-3.31- -0.62) *	-0.27(-1.61-1.08)	-0.47(-1.81-0.88)
63rd day	0	-0.07(-0.85-0.72)	-0.03(-0.75-0.82)	-0.63(-1.42-0.15)
70th day	0	0.03(-0.43-0.49)	0.10(-0.36-0.56)	-0.07(-0.53-0.39)

*Notes: Using one-way ANOVA and Tukey test on post-hoc. * indicates significance (p<0.05).*

Key: *Hb A_{1C}=Haemoglobin A_{1C}*

4.5 Effects of *Cucumis metuliferus* Fruit Extract on Fasting Blood Sugar

There was a significant statistical rise in fasting blood sugar (FBS) between the control group and the treatment groups on days 42 (P <0.001), day 45 (P <0.001), and day 56 (P = 0.030), but no significant statistical difference on days 63 (P =0.090) and 70 (P =0.163) of the experiment. Post hoc statistical analysis using the Tukey test revealed a significant rise (P <0.001) in FBS in the positive control group (5.76±0.901 test vs. 2.80±0.608 control), low dose CMFE (5.43±0.447 test vs. 2.80±0.608 control), and high dose CMFE (5.57±0.682 test vs. 2.80±0.608 control) on day 42 of the experiment. Likewise, there was a significant rise (P <0.001) in FBS in the positive control group (13.96±2.790 test 3.37±1.011control), low dose CMFE (11.80±2.890 test vs. 3.37±1.011 control), and high dose CMFE (12.52±2.892 test vs. 3.37±1.011 control) on day 45 of the experiment. A significantly high (P = 0.030) FBS was also noted in the positive control group (6.53±1.527 test vs. 2.96±0.551 control) on day 56 of the experiment.

The rise in FBS observed on day 42 could be attributed to treatment with a high-fat and fructose diet for 6 weeks before treatment with STZ to induce type II DM. However, the increase in FBS observed on day 45 demonstrates the successful induction of type II DM after treatment with STZ. The increase in FBS in the positive control group on day 56 indicates that treatment with CMFE achieved early FBS control as far as treatment duration is concerned. Comparable levels of FBS observed on days 63 and 70 of the experiment confirm that CMFE controls blood sugar (Table 4.5).

Table 4.5: Comparison of mean Fasting Blood Sugar between Various Treatment Groups

Test Day	Control	Positive Control –PGZ (20mg/kg)	Low dose CMFE 200mg/kg	High dose CMFE (400mg/kg)	F	*p*-value
42nd day	2.80±0.608	5.76±0.901*	5.43±0.447*	5.57±0.682*	14.775	<0.001
45th day	3.37±1.011	13.96±2.790*	11.80±2.890*	12.52±2.892*	11.390	<0.001
56th day	2.96±0.551	6.53±1.527*	4.40±1.015	5.37±1.320	5.061	0.030
63rd day	3.33±0.379	4.40±0.200	2.80±0.794	4.30±0.300	7.945	0.090
70th day	3.067±1.185	3.77±0.351	4.57±0.252	4.00±0.700	2.224	0.163

Tukey test on post-hoc (mean difference)

Test Day	Control	Positive Control –PGZ (20mg/kg)	Low dose CMFE 200mg/kg	High dose CMFE (400mg/kg)
42nd day	0	-2.95(-4.227- -1.684) *	-2.63(-3.905- -1.362) *	-2.77(-4.038- -1.495) *
45th day	0	-10.60(-15.648- -5.552) *	-8.44(-13.841- -3.386) *	-9.16(-14.203- -4.108) *
56th day	0	-3.57(-6.608- -0.526) *	-1.43(-4.474- 1.608)	-2.40(-5.441- 0.608)
63rd day	0	-1.07(-2.981- 0.847)	0.53(-1.381-2.447)	-0.967(-2.902- 0. 276)
70th day	0	-0.70(-2.586-1.186)	-1.50(-3.386-0.386)	-0.93(-2.819- 1.652)

*Notes: Using one-way ANOVA and Tukey test on post-hoc. * indicates significance (p<0.05).*

Key: *FBS=Fasting Blood Glucose*

4.6 Effects of *Cucumis metuliferus* Fruit Extract on Oral Glucose Tolerance Test

There was a significant statistical rise in OGTT between the control group and the treatment groups on days 42 (P <0.001), day 45 (P <0.001), day 56 (P =0.002), and day 63 (P = 0.039), but no significant statistical difference (P =0.106) on day 70 of the experiment. Post hoc statistical analysis using the Tukey test revealed significantly high (P <0.001) OGTT levels in all treatment groups on day 45 of the experiment. The rise

could be attributed to insulin resistance following treatment with a high-fat and fructose diet for 6 weeks before treatment with STZ to induce type II DM. There was a significant statistical increase ($P < 0.001$) in OGTT levels in all treatment groups on day 45. The rise observed on day 45 indicates successful induction of type II DM following treatment with STZ. However, there was a significant statistical increase ($P = 0.002$) in OGTT in the group treated with 20mg/kg PGZ (10.26±2.424 test vs. 3.46±0.115 control) and in the group treated with high dose CMFE (9.76±3.102 test vs. 3.46±0.115 control) on day 56 of the experiment. The test results on day 56 of the experiment indicate poor blood glucose control in the positive control group and the group treated with high-dose CMFE following an oral glucose tolerance test. There was also a significant statistical increase ($P = 0.036$) in OGTT in the group treated with high dose CMFE (8.64±0.417 test vs. 3.10±0.100 control) on day 63 of the experiment, indicative that this group had poor glucose control following an oral glucose tolerance test. However, the decrease in OGTT in the treatment groups to levels comparable to the control group with no significant difference ($P = 0.106$) on day 70 of the experiment indicates that CMFE controls the blood sugar in the oral glucose tolerance test (Table 4.6).

Table 4.6: Comparison of mean Blood Sugar in Oral Glucose Tolerance Test between various treatment groups

Test Day	Control	Positive Control –PGZ (20mg/kg)	Low dose CMFE 200mg/kg	High dose CMFE (400mg/kg)	F	P-value
42nd day	3.69±0.469	6.44±0.621*	6.17±0.335*	6.72±0.450*	16.154	<0.001
45th day	3.71±0.364	17.01±0.791*	13.39±1.326*	15.42±0.290*	48.850	<0.001
56th day	3.46±0.115	10.26±2.424*	7.13±0.911	9.76±3.102*	12.533	0.002
63rd day	3.10±0.100	8.217±3.945	5.525±1.151	8.64±0.417*	4.679	0.036
70th day	5.06±0.317	6.33±0.893	7.08±0.439	5.90±1.494	2.833	0.106

Tukey test on post-hoc (mean difference)

Days	Control	Positive Control –PGZ (20mg/kg)	Low dose CMFE 200mg/kg	High dose CMFE (400mg/kg)
42nd day	0	-2.75(-4.001- -1.499) *	-2.48(-3.732- -1.223) *	-3.03(-4.28- -1.774) *
45th day	0	-13.30(-15.409- -11.192) *	-9.68(-11.78- -7.569) *	-11.71(-13.82- -9.603) *
56th day	0	-6.80(-10.784- -2.816) *	-3.67(-7.651- 0.317)	-6.30(-10.283- -2.316) *
63rd day	0	-5.12(-11.505-1.271)	-2.42(-8.813-3.963)	-5.54(-10.942- -0.138)*
70th day	0	-1.28(-3.542-0.992)	-2.02(-4.284- 0.251)	0.84(-3.109-1.426)

*Notes: Using one-way ANOVA and Tukey test on post-hoc. * indicates significance (p<0.05).*

Key: *Oral Glucose Tolerance Test*

4.7 Effects of *Cucumis metuliferus* Fruit Extract on Lipid Profile

There was a significant statistical increase (P = 0.019) in serum triglycerides (TG) between the control group and the treatment groups on day 45 of the experiment, with no significant statistical difference on days 56 (P =0.478), day 63 (P = 0.188), and day 70 (P =0.420) of the experiment. Post hoc statistical analysis using the Tukey statistical test revealed a significant rise (P =0.019) in serum triglycerides in all treatment groups on day 45 of the experiment.

There was a significant statistical increase (P <0.001) in total cholesterol (TC) between the control group and the treatment groups on day 45, with no significant statistical difference on days 56 (P =0.180), day 63 (P = 0.193), and day 70 (P =0.104) of the

experiment. Post hoc statistical analysis using the Tukey test revealed a significant increase (P <0.001) in TC in all the treatment groups on day 45 of the experiment.

There was a significant statistical decrease (P = 0.004) in serum high-density lipoprotein (HDL) between the control group and the treatment groups on day 45, with a significant statistical increase (P =0.001) on day 70 of the experiment. However, there was no statistical difference on days 56 (P =0.671) and 63 (P =0.583) of the experiment. Post hoc statistical analysis using the Tukey statistical test revealed a significant decrease (P =0.004) of serum HDL in all the treatment groups on day 45 of the experiment. However, the test also revealed a significant rise in serum HDL in the group treated with low dose CMFE (1.47±0.58 test vs. 1.27±0.058 control) and in the group treated with high dose CMFE (1.50±0.100 test vs. 1.27±0.058 control) on day 70 of the experiment.

There was a significant statistical increase (P = 0.016) in serum low-density lipoprotein (LDL) between the control group and treatment groups on day 45, with a significant statistical decrease (P = 0.003) on day 56 of the experiment. However, there was no significant statistical difference on days 63 (P =0.126) and 70 (P =0.793) of the experiment. Post hoc statistical analysis using the Tukey test revealed a significant increase (P =0.016) in serum LDL in all the treatment groups on day 45 of the experiment. A significant drop in serum LDL was observed in the positive control group (0.20±0.100 test vs. 0.67±0.058 control), low dose CMFE (0.33±0.153 test vs. 0.67±0.058 control), and high dose CMFE (0.23±0.115 test vs. 0.67±0.058 control) on day 56 of the experiment (Table 4.8).

An increase in serum triglycerides, total cholesterol, and low-density lipoproteins and a decrease in serum high-density lipoproteins in the treatment groups on day 45 of the experiment could be attributed to treatment with a high-fat and fructose diet for six weeks before treatment with STZ to induce type II DM. Contrary to this, the decrease of serum triglycerides, total cholesterol, and low-density lipoproteins in treatment groups to levels comparable to the control group and the increase of serum high-density lipoproteins

thereafter confirm that indeed CMFE corrected nutritionally induced dyslipidaemia and prevented diabetic-associated dyslipidaemia (Table 4.7).

Table 4.7: Comparison of Lipid Profile between Various Treatment Groups

	Test Day	**Control**	**Positive Control-PGZ (20mg/kg)**	**Low Dose CMFE (200mg/kg)**	**High Dose CMFE (400mg/kg)**	**F**	***p* value**
TG	45	0.67±0.058	1.20±0.300*	1.17±0.115*	1.17±0.153*	6.051	0.019
	56	0.77±0.153	0.90±0.265	0.93±0.145	0.67±0.208	0.911	0.478
	63	0.70±0.100	1.27±0.252	1.13±0.321	1.20±0.458	2.032	0.188
	70	0.87±0.153	1.27±0.306	1.23±0.416	1.37±0.503	1.055	0.420
TC	45	1.20±0.100	2.77±0.153*	2.73±0.208*	2.70±0.100*	81.487	<0.001
	56	1.50±0.108	1.40±0.361	1.93±0.416	1.53±0.058	2.088	0.180
	63	1.57±0.208	1.93±0.208	1.73±0.153	1.63±0.208	2.000	0.193
	70	1.67±0.058	2.10±0.361	1.90±0.100	1.77±0.580	2.871	0.104
HDL	45	1.37±0.115	0.90±0.100*	0.73±0.208*	0.80±0.173*	10.161	0.004
	56	1.30±0.100	1.10±0.400	1.37±0.379	1.37±0.208	0.536	0.671
	63	1.17±0.153	1.27±0.153	1.30±0.200	1.33±0.058	0.691	0.583
	70	1.27±0.058	1.17±0.058	1.47±0.058 *	1.50±0.100*	15.333	0.001
LDL	45	0.60±0.100	1.10±0.100*	1.07±0.153*	1.07±0.252*	6.448	0.016
	56	0.67±0.058	0.20±0.100*	0.33±0.153*	0.23±0.115*	10.911	0.003
	63	0.73±0.153	0.50±0.100	0.60±0.173	0.47±0.058	2.583	0.126
	70	0.70±0.200	0.77±0.252	0.83±0.493	0.97±0.321	0.346	0.793

Post Hoc test (Mean difference)

	Test Day	**Control**	**Positive control PGZ (20mg/kg)**	**Low Dose CMFE (200mg/kg)**	**High Dose CMFE (400mg/kg)**
TG	45	0	0.53(0.06-1.00)*	0.50(0.30-0.97)*	0.50(0.03-0.98)*
	56	0	0.13(-0.45- 0.72)	0.17(-0.42-0.75)	0.10 (-0.69 - 0.48)
	63	0	0.57(-0.25-1.38)	0.43(-0.38-1.25)	0.50(-0.31- 1.31)
	70	0	0.40(-0.78-1.58)	0.37(-0.81-1.54)	0.50(-0.46-1.46)
TC	45	0	1.57(1.18 – 1.95) *	1.53(1.15– 1.92) *	1.50(1.12 – 1.88) *
	56	0	-0.10(-0.84-0.64)	0.43(-0.30-1.17)	0.03(-0.70-0.77)
	63	0	-0.37(-0.90-0.16)	-0.57(-1.10- -0.04) *	-0.57(-1.10- -0.04) *
	70	0	0.43(-0.07-0.92)	0.23(-0.27-0.730)	0.10(-0.40-0.60)
HDL	45	0	1.07 (0.33 – 1.80) *	1.12(0.39 – 1.86) *	1.10 (0.37 – 1.83)*
	56	0	0.20(-0.58-0.98)	0.07(-0.85-0.71)	0.07(-0.71-0.85)
	63	0	0.10(-0.29-0.49)	0.13(-0.30-0.526)	0.17(-0.23-0.56)
	70	0	- 0.10(-0.29-0.09)	0.20(-0.02 - 0.38) *	0.23(0.05-0.421) *
LDL	45	0	0.50(0.07- 0.93)*	0.46 (0.04 – 0.89)*	0.46 (0.04 – 0.89)*
	56	0	- 0.47(-0.76- - 0.17) *	- 0.33(-0.63- -0.04) *	-0.43(-0.73 - -0.14) *
	63	0	- 0.23(-0.57-0.10)	- 0.13(-0.47-0.21)	- 0.27(-0.60-0.07)
	70	0	0.07(-0.81-0.94)	0.13(-0.74-1.01)	0.27(-0.61 -1.14)

*Notes: Using one-way ANOVA and Tukey test on post-hoc. * indicates significance (p<0.05).*

Key: *TG=Triglycerides; TC=Total Cholesterol; HDL=High Density Lipoprotein; LDL=Low Density Lipoprotein*

4.8 Effects of *Cucumis metuliferus* Fruit Extract on Liver Enzymes

There was a significant statistical rise in alanine aminotransferase (ALT) between the control group and the treatment groups on days 45 ($P < 0.001$) and 56 ($P = 0.001$), but no significant statistical difference on days 63 ($P = 0.193$) and 70 ($P = 0.661$) of the experiment. Post hoc statistical analysis using the Tukey test revealed a significant statistical rise ($P < 0.001$) of serum ALT in all treatment groups on day 45 of the experiment. The test also revealed a significant statistical rise of serum ALT in the group treated with low dose CMFE (77.9±7.78 test vs. 22.3±1.52 control) and in the group treated with high dose CMFE (73.4±21.45 test vs. 22.3±1.52 control) on day 56 of the experiment.

There was a significant statistical rise in serum aspartate aminotransferase (AST) between the control group and the treatment groups on days 45 ($P < 0.001$) and 56 ($P < 0.001$), with a significant statistical decrease ($P < 0.001$) on day 63 of the experiment. However, there was no significant statistical difference ($P = 0.123$) on day 70 of the experiment. Post hoc statistical analysis using the Tukey test showed a significant rise in serum AST in all the treatment groups on days 45 ($P < 0.001$) and 56 ($P < 0.001$) of the experiment. However, a significant decrease ($P < 0.001$) in serum AST was observed in the group treated with low-dose CMFE (17.2±3.01 test vs. 29.3±1.53 control) and in the group treated with high-dose CMFE (15.2±2.01 test vs. 29.3±1.53 control) on day 63 of the experiment.

There was a significant statistical increase in gamma-glutamyl transferase (GGT) between the control group and the treatment groups on days 45 ($P = 0.002$), 56 ($P = 0.015$), and 70 ($P = 0.015$), with no significant statistical difference ($P = 0.193$) on day 63 of the experiment. Post hoc statistical analysis using the Tukey test revealed a significant increase in serum GGT in all the treatment groups on day 45 of the experiment, in the group treated with high dose CMFE (65.27±1.29 test vs. 1.1±0.10 control) and in the positive control group (4.77±1.90 test 1.1±0.10 control) and (5.13±1.53 test vs. 1.17±0.06 control) on days 56 and 70, respectively, of the experiment.

There was no significant difference in alkaline phosphatase (ALP) between the control group and the treatment groups on days 45 (P = 0.050), 56 (P = 0.226), 63 (P = 0.524), and 70 (P = 0.415) of the experiment. (Table 4.9)

Increased levels of serum ALT, AST, and GGT on day 45 of the experiment could be attributed to streptozotocin (STZ)-induced liver damage following treatment with STZ to induce type II DM, and the decrease observed thereafter and at the end of the experiment in the treatment groups to levels comparable to the control group confirmed that indeed CMFE restored liver enzymes (Table 4.8).

Table 4.8: Comparison of Liver Enzymes Between Various Treatment Groups

	Test Day	**Control**	**Positive Control-PGZ (20mg/kg)**	**Low Dose CMFE (200mg/kg)**	**High Dose CMFE (400mg/kg)**	**F**	**P-value**
ALT	45	19.00±1.00	56.33±8.61*	97.90±7.99*	108.87±18.17*	43.293	<0.001
	56	22.3±1.53	37.5±5.17	77.9±7.78*	73.4±21.45*	16.11	0.001
	63	19.0±1.00	26.9±11.25	34.5±11.62	32.10±4.44	1.995	0.193
	70	23.7±0.58	22.7±8.02	18.67±8.96	18.47±3.48	0.552	0.661
AST	45	25.00±3.00	93.50±7.14*	115.93±7.09*	112.80±7.41*	130.581	<0.001
	56	28.7±1.53	70.2±18.58*	92.4±2.91*	86.97±9.00*	29.697	<0.001
	63	29.3±1.53	33.1±3.33	17.2±3.01*	15.2±2.01*	17.029	<0.001
	70	28.3±5.77	32.0±1.73	17.6±9.11	23.83±7.57	2.614	0.123
GGT	45	1.17±0.15	5.90±1.54*	9.000±0.17*	7.4±2.86*	12.912	0.002
	56	1.10±0.10	4.77±1.90*	4.17±1.05	5.27±1.29*	6.588	0.015
	63	1.07±0.06	4.53±3.44	2.53±1.12	4.67±5.52	1.278	0.460
	70	1.17±0.06	5.13±1.53*	2.33±0.93	3.50±1.44	6.505	0.015
ALP	45	65.67±3.51	83.00±4.58	92.33±11.91	82.67±18.88	13.615	0.050
	56	65.67±3.51	70.00±3.61	54.00±6.00	60.67±10.81	1.794	0.226
	63	67.33±5.68	62.33±3.79	56.67±7.57	63.00±13.45	0.808	0.524
	70	57.37±5.69	52.33±2.61	46.36±6.96	53.00±11.46	0.701	0.415

Post Hoc test (Mean difference)

	Test Day	Control	Positive Control-PGZ (20mg/kg)	Low Dose CMFE (200mg/kg)	High Dose CMFE (400mg/kg)
ALT	45	0	37.33(9.02 – 65.64) *	78.90(50.59 – 107.29) *	89.87(61.56 – 118.18)*
	56	0	15.17(-15.49-45.82)	55.53(24.88-86.19) *	51.07(20.41 – 81.72)*
	63	0	7.90(-14.07 - 29.87)	15.47(-6.50 - 37.43)	13.10(-8.87 - 35.07)
	70	0	-1.00(-17.39 - 15.39)	-5.00(-21.39-11.39)	-5.20(-21.59 - 11.19)
AST	45	0	68.50(51.70 – 85.30) *	90.93(74.14 – 107.73) *	87.80(71.00 – 104.60) *
	56	0	41.57 (14.24 - 68.89) *	63.70(36.37 – 91.03) *	58.30 (30.97 – 85.63)*
	63	0	3.80(-6.30 – 13.90)	-12.13(-22.4 - -2.03) *	-15.20(-25.30- -5.10)*
	70	0	3.67(-14.94 - 22.27)	-10.73(-29.34-7.87)	-4.50(-21.88-12.88)
GGT	45	0	4.733(0.47 – 8.99) *	7.83 (3.57 – 12.09) *	6.23 (0.007 – 1.97) *
	56	0	3.67(0.36 - 6.97)*	3.07(-0.24 – 6.37)	4.17(0.86- 7.47)*
	63	0	3.47(-3.44 – 10.38)	1.47(-5.44 – 8.38)	3.60 (-3.31 – 10.51)
	70	0	3.97(0.96 -6.97) *	-1.17(-1.84-4.17)	2.33(-0.67-5.34)
ALP	45	0	20.67 (-9.47-50.81)	30.00 (-0.14 – 60.14)	20.33(-9.81 – 50.47)
	56	0	4.33(-22.80 – 31.46)	-11.67(-38.80 – 15.46)	- 5.00(-32.13 – 20.46)
	63	0	-1.00 (-10.93- 8.93)	-2.67(-12.59- 7.26)	-6.67(-16.59 - 3.26)
	70	0	-5.00(-27.07-17.07)	-10.67(-32.74-11.40)	-4.33(-26.40-17.74)

*Notes: Using one-way ANOVA and Tukey test on post-hoc. * indicates significance (p<0.05).*

Key: *ALT= Alananine aminotransferase; AST=Aspartate aminitransferase; GGT=Gamma glutamyl transferase; ALP=Alkaline phosphatase*

4.9 Effects of *Cucumis metuliferus* Fruit Extract on Serum Proteins

There was a significant increase in serum total proteins (TP) between the control group and treatment groups on days 63 (P = 0.002) and 70 (P = 0.023), with no significant statistical difference on days 45 (P = 0.169) and 56 (P = 0.608) of the experiment. Post hoc statistical analysis using the Tukey test revealed a significant increase (P = 0.002) in serum TP in the group treated with high dose CMFE (80.17±1.02 test vs. 69.00±1.05 control) and (80.67±1.26 test vs. 72.33 ± 3.55 control) on days 63 and 70, respectively, of the experiment.

There was a significant statistical rise in serum albumin (ALB) between the control group and treatment groups on days 56 (P =0.024), 63 (P < 0.01), and 70 (P = 0.001), with no significant statistical difference (P = 0.194) on day 45 of the experiment. Post hoc statistical analysis using the Tukey test revealed a significant increase (P = 0.024) in serum ALB in the group treated with high dose CMFE (40.40±1.51 test vs. 31.70±1.54 control) on day 56 of the experiment. Likewise, there was a significant rise (P< 0.01) of mean serum albumin in the group treated with low dose CMFE (38.43±0.81 test vs. 34.03±1.71

control) and in the group treated with high dose CMFE (46.90±1.53 test vs. 34.03±1.71 control) on day 63 of the experiment. The Tukey test also revealed a statistical rise (P = 0.001) of serum albumin in the group treated with high-dose CMFE (49.27±3.10 test vs. 38.67±1.44 control) on day 70 of the experiment. These results show that treatment with CMFE increased the synthesis of albumin and overall total proteins in the liver and their availability in the blood (Table 4.9).

Table 4.9: Comparison of Serum Proteins between Various Treatment Groups

	Test Day	Control	Positive Control-PGZ (20mg/kg)	Low Dose CMFE (200mg/kg)	High Dose CMFE (400mg/kg)	F	P-value
TP	45	64.77±4.80	70.93±4.90	64.70±4.52	55.03±15.59	1.814	0.169
	56	67.23±1.73	71.57±8.79	69.07±3.40	60.47±18.15	0.644	0.608
	63	69.00±1.05	70.63±1.52	65.60±5.82	80.17±1.02*	12.152	0.002
	70	72.33±3.55	67.03±4.57	73.13±5.69	80.67±1.26*	5.603	0.023
ALB	45	31.70±2.65	31.67±3.11	32.07±2.10	31.90±2.30	1.690	0.194
	56	31.70±1.54	32.50±4.90	36.87±2.64	40.40±1.51*	5.524	0.024
	63	34.03±1.71	35.13±0.47	38.43±0.81	46.90±1.53*	65.882	< 0.01
	70	38.67±1.44	32.60±4.43	38.37±1.61	49.27±3.10*	17.057	0.001

Post Hoc test (Mean difference)

	Test Day	Control	Positive Control-PGZ (20mg/kg)	Low Dose CMFE (200mg/kg)	High Dose CMFE (400mg/kg)
TP	45		6.17(-16.87 – 29.20)	- 0.07(-23.10 - 22.97)	-9.73(-32.77 – 13.30)
	56	0	4.33(– 22.503 - 31.169)	1.83(– 25.003 - 28.670)	-6.77(– 33.603 - 20.070)
	63	0	1.63(– 6.474 - 9.741)	-3.40(– 11.507 - 4.704)	11.17(3.059-19.274-) *
	70	0	-5.30(– 16.038 - 5.438)	0.80(– 9.938 - 11.538)	8.33(2.405 - 19.074)*
ALB	45	0	-0.03(-6.675 – 6.68)	0.37(-6.32-7.12)	0.20(-6.522 – 6.923)
	56	0	0.80(–7.007 - 8.604)	5.17(–2.641 - 12.974)	8.70(0.891 – 16.513) *
	63	0	1.10(– 2.150 - 4.350)	4.40(1.150 -7.650)*	12.87(9.617-16.117) *
	70	0	-6.07(-13.684 - 1.551)	-0.30(-7.912 - 7.318)	10.60(2.983 - 18.218) *

*Notes: Using one-way ANOVA and Tukey test on post-hoc. * indicates significance (p<0.05*

Key: *TP= Total Protein* *ALB=Albumin*

4.10 Effects of *Cucumis metuliferus* Fruit Extract on Serum Bilirubin

There was a significant statistical decrease (P = 0.031) in total bilirubin (TBIL) between the control group and treatment groups on day 45, with no statistical difference on days

56 (P = 0.844), 63 (P = 0.295), and 70 (P = 0.479) of the experiment. Post hoc statistical analysis using the Tukey test revealed a significant decrease (P = 0.031) of serum TBIL in the group treated with low-dose CMFE (1.53±0.25 test vs. 5.47±2.12 control) on day 45 of the experiment.

There was no significant statistical difference in serum direct bilirubin (DBIL) between the control group and treatment groups on days 45 (P = 0.194), 56 (P = 0.423), 63 (P = 0.179), and 70 (P = 0.256) of the experiment.

The decrease in serum total bilirubin observed on day 45 of the experiment could be attributed to streptozotocin (STZ)-induced liver damage following treatment with STZ to induce type II DM. However, comparable levels of serum total bilirubin between the control and treatment groups thereafter and direct bilirubin throughout the experiment suggest that CMFE has hepato-restorative properties (Table 4.10).

Table 4.10: Comparison of Serum Bilirubin Between Various Treatment Groups

	Test Day	**Control**	**Positive Control-PGZ (20mg/kg)**	**Low Dose CMFE (200mg/kg)**	**High Dose CMFE (400mg/kg)**	**F**	**P-value**
TBIL	45	3.70±0.53	5.47±2.12	1.53±0.25*	4.00±1.21	5.005	0.031
	56	4.17±0.64	4.10±1.35	3.50±0.60	4.2±1.49	0.272	0.844
	63	4.50±0.61	3.83±1.35	3.50±0.35	3.77±0.45	1.126	0.295
	70	4.70±0.66	5.23±1.42	4.00±0.70	4.60±0.92	0.908	0.479
DBIL	45	2.33±0.21	2.80±0.79	1.87±0.40	2.63±0.42	1.992	0.194
	56	2.37±0.21	2.87±.45	1.77±0.42	2.77±0.19	0.583	0.423
	63	2.53±0.40	2.37±0.75	2.27±0.06	2.50±0.60	2.094	0.179
	70	2.77±0.32	2.77±0.40	2.23±0.12	2.87±0.57	1.641	0.256

Post Hoc test (Mean difference)

	Test Day	Control	Positive Control-PGZ (20mg/kg)	Low Dose CMFE (200mg/kg)	High Dose CMFE (400mg/kg)
TBIL	45	0	1.77(-1.521 – 5.050)	-2.17(-5.453 – -1.122) *	0.30(-2.992-3.591)
	56	0	-0.07(-2.931- 2.833)	-0.67(-3.534-6.2.202)	0.03(-2.834-2.900)
	63	0	-0.67(-2.74-1.41)	-1.00(-3.07-1.07)	-0.73(-2.81-1.34)
	70	0	0.53(-1.90-2.97)	-0.70(-3.14-1.74)	-0.23(-2.67-2.20)
DBIL	45	0	0.47 (-0.851 – 1.776)	-0.47(-1.781 – 0.853)	0.30(-1.014 – 1.611)
	56	0	0.50(-0.451 – 1.452)	-0.60(-1.553-0.354)	0.40(-0.552-1.352)
	63	0	-0.17(-1.53-1.20)	-0.27(-1.63-1.10)	0.03(-1.40-1.60)
	70	0	0.01(-1.02-1.02)	0.53(-1.55-0.48)	0.10(-0.92- 1.12)

*Notes: Using one-way ANOVA and Tukey test on post-hoc. * indicates significance (p<0.05).*

Key: *TBIL=Total Bilirubin* *DBIL=Direct Bilirubin*

4.11 Effects of *Cucumis metuliferus* Fruit Extract on Body Weight

There was a significant statistical increase (P = 0.033) in mean body weight between the control group and treatment groups on day 42 of the experiment. However, there was a significant statistical decrease in mean body weight between the control group and treatment groups on days 56 (P <0.001), 63 (P <0.001), and 70 (P <0.001), with no significant statistical difference (P = 0.479) on day 49 of the experiment. Post hoc statistical analysis using the Tukey test revealed a significant statistical increase (P = 0.033) in mean body weight in all the treatment groups on day 42 of the experiment. However, there was a significant statistical decrease (P <0.001) of mean body weight in the positive control group (168.07±13.264 test vs. 266.10±12.272 control) and in the group treated with high dose CMFE (186.11±9.279 test vs. 266.10±12.272 control) on day 56 of the experiment. Likewise, there was a significant statistical decrease in mean body weight in all treatment groups on days 63 (P <0.001) and 70 (P <0.001) of the experiment.

An increase in total body weight on day 42 of the experiment could be attributed to treatment with a high-fat and fructose diet for 6 weeks prior to treatment with STZ to induce type II DM. A drop in weight observed in the treatment groups on days 56, 63, and 70 of the experiment could be due to disease progression and its effect on total metabolic functions in type II DM (Table 4.11).

Table 4.11: Comparison of Mean Body Weights between Various Treatment Groups

Test Day	Control	Positive Control-PGZ (20mg/kg)	Low Dose CMFE (200mg/kg)	High Dose CMFE (400mg/kg)	F	Control
1 day	97.40±4.060	102.48±4.022	107.94±7.270	107.59±5.812	2.489	0.134
7th day	113.40±3.617	125.26±9.968	128.34±10.103	127.98±4.58	2.516	0.132
14th day	132.70±6.194	150.00±16.056	157.31±12.730	154.98±5.074	3.068	0.091
21st day	164.33±4.350	179.41±18.101	189.82±13.358	175.76±12.756	1.930	0.203
28th day	174.90±9.071	206.27±23.256	207.94±13.355	202.91±14.534	3.946	0.054
35th day	198.10±8.730	224.33±22.415	237.49±14.612	220.77±14.334	3.227	0.082
42nd day	216.53±8.083	242.43±21.439*	258.30±18.455*	239.84±13.150*	3.426	0.033
49th day	237.27±9.692	225.44±24.348	245.29±19.769	225.78±12.059	0.908	0.479
56th day	266.10±12.272	168.07±13.264*	269.06±20.998	186.11±9.279*	39.153	<0.001
63rd day	294.80±10.065	208.25±27.291*	186.69±2.216*	205.40±23.285*	20.093	<0.001
70th day	320.43±11.859	214.67±14.635*	231.27±14.371*	195.25±22.412*	34.557	<0.001

Tukey test on post-hoc (mean difference)

Test Day	Control	Positive Control-PGZ (20mg/kg)	Low Dose CMFE (200mg/kg)	High Dose CMFE (400mg/kg)
0 day		-5.08(-19.356-9.2004)	-10.54(-24.823-3.734)	-10.19(-24.467-4.089)
7th day		-11.86(-31.936-8.225)	-14.94(-35.025-5.136)	-14.58(-34.658-5.503)
14th day		-17.27(-46.027-11.494)	-24.61(53.371-4.150)	-22.28(-51.038-6.483)
21st day		-15.08(-49.363-19.208)	-25.489(-59.774-8.797)	-11.42(-45.708-22.863)
28th day		-31.37(-72.97-10.234)	-43.04(-84.645- 1.444)	-28.03(-69.634-13.568)
35th day		-26.23(-67.526-15.059)	-39.39(-80.682-1.904)	-22.67(-63.959-18.626)
42nd day		-25.90(-68.030--16.230)*	-41.77(-83.897- -0.364) *	-23.31(-65.442- -8.819) *
49th day		11.82(-3.898-57.543)	-8.02(-53.743-37.698)	11.49(-34.232-57.209)
56th day		98.03(59.831-136.221) *	-2.96(-41.150-35.239)	79.99(41.794-118.184) *
63rd day		86.55(37.756-135.351) *	108.11(59.31-156.905) *	89.40(40.599-138.195) *
70th day		105.76(63.122-148.40) *	89.17(46.528-131.803) *	125.19(82.55-167.827) *

4.12 Effects of *Cucumis metuliferus* Fruit Extract on the Histomorphology of the Islet of Langerhans

There was a uniform distribution of the cells in the Islet of Langerhans, with a homogenous distribution of connective tissue matrix and blood vessels in the control group (Figure 4.1). A marked reduction of cell density in the Islet of Langerhans was observed in the negative control group (Induced DM but treated with normal saline). The cells had pyknotic nuclei and were less basophilic when compared to both the control and the positive control groups (Figure 4.2). The positive control group (20mg/kg PGZ) demonstrated a sparse population of cells in the Islet of Langerhans, which was however basophilic when compared to the control group (Figure 4.3). The low-dose CMFE group demonstrated cell distribution comparable to that of the control group but with a lesser cell population than the positive control (Figure 4.4). The high-dose CMFE group (400mg/kg) had more basophilic nuclei when compared to the control, negative control, and positive control groups (Figure 4.5). Variation of the endocrine pancreas histomorphology in different treatment groups is shown in figure 4.6

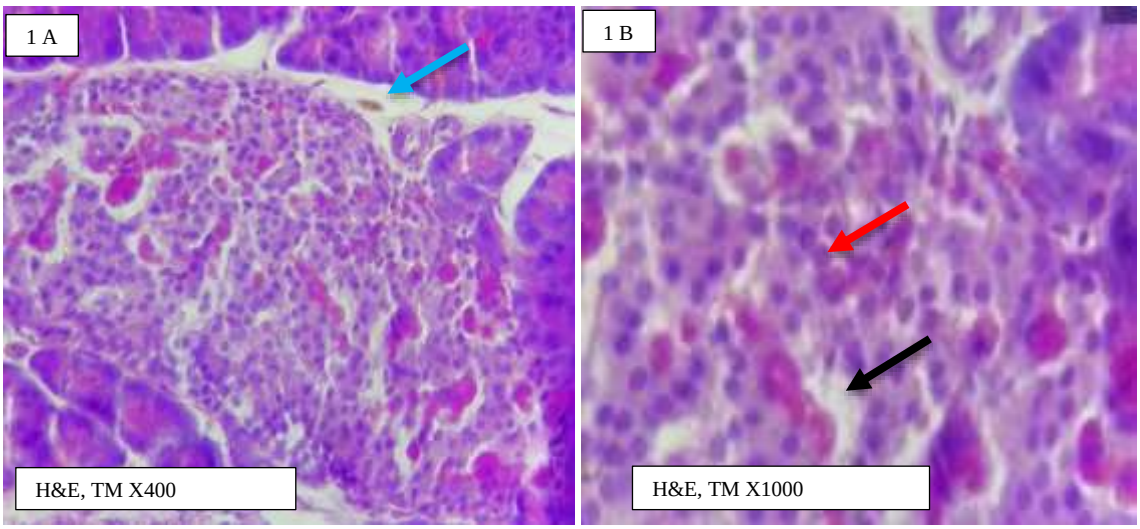

Figure 4.1: Photomicrographs of Endocrine Pancreas Histomorphology in the Control Group at x400 (1A) and x1000 (1B) Magnifications.

Key

"Red arrow" Islet cell

"Black arrow" Connective tissue

"Blue arrow" Interlobular septum

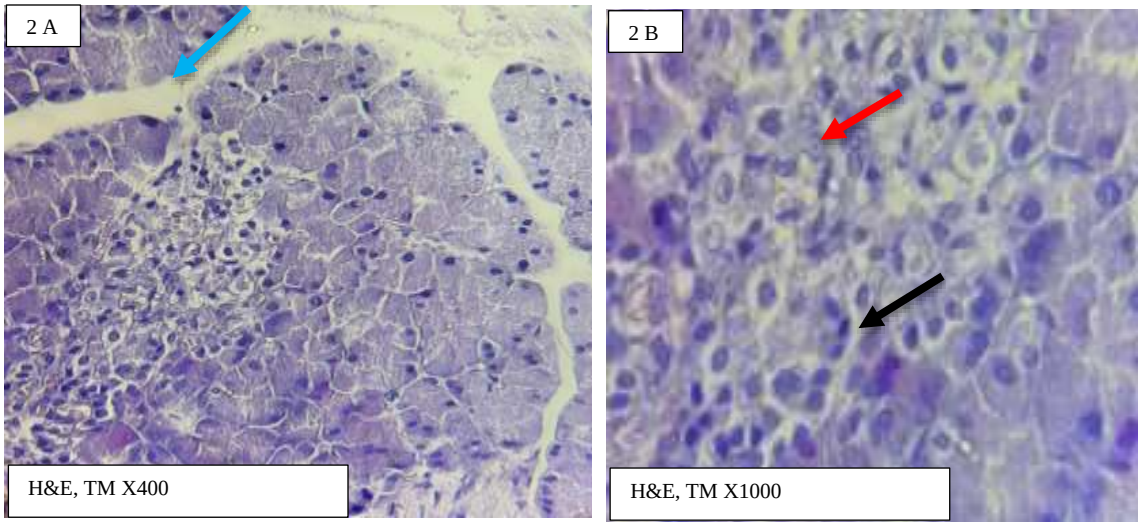

Figure 4.2: Photomicrographs of Endocrine Pancreas Histomorphology in the Negative Control Group at x400 (2A) and x1000 (2B) Magnifications

Key

"Red arrow" Islet cell

"Black arrow" Connective tissue

"Blue arrow" Interlobular septum

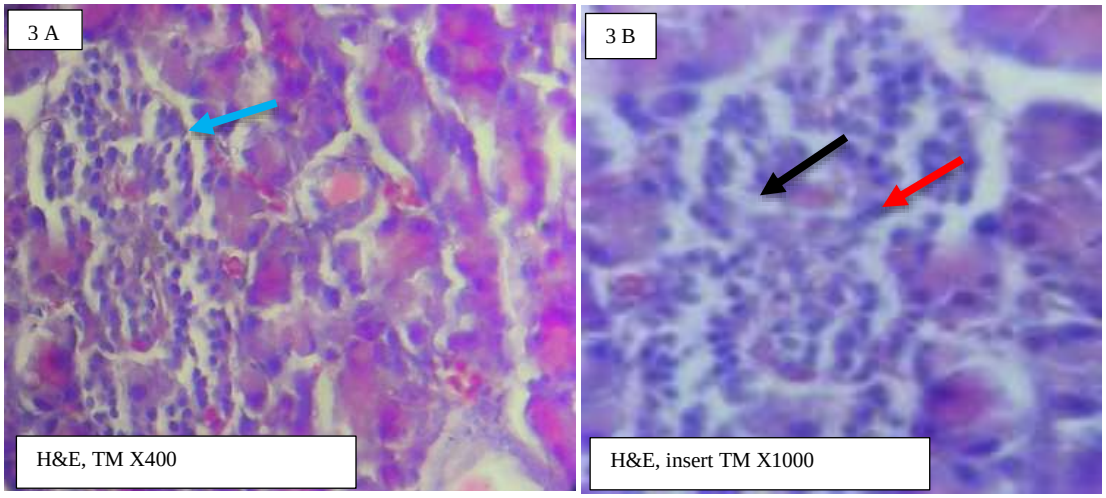

Figure 4.3: Photomicrographs of Endocrine Pancreas Histomorphology in the Positive Control Group at x400 (3A) and x1000 (3b) Magnifications

Key

"Red arrow" Islet cell

"Black arrow" Connective tissue

"Blue arrow" Interlobular septum

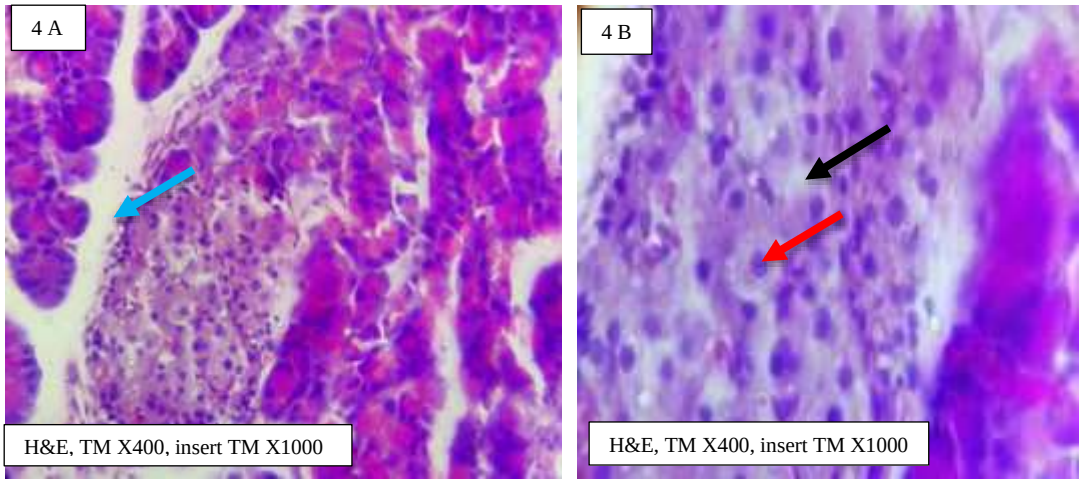

Figure 4.4: Photomicrographs of Endocrine Pancreas Histomorphology in the Low-Dose CMFE at x400 (4A) and x1000 (4B) Magnifications.

Key

"Red arrow" Islet cell

"Black arrow" Connective tissue

"Blue arrow" Interlobular septum

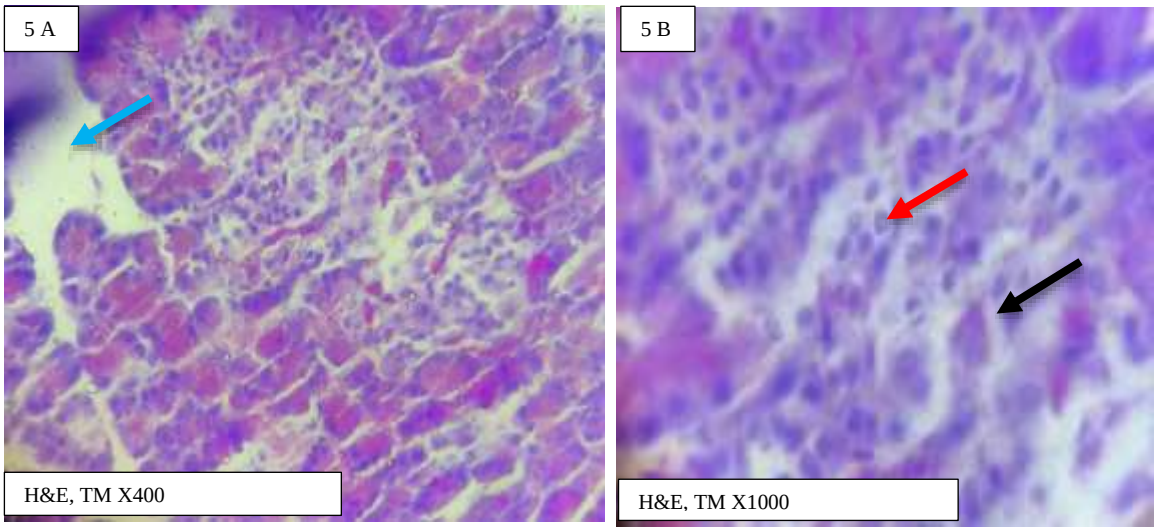

Figure 4.5: Photomicrographs of Endocrine Pancreas Histomorphology in the High-Dose CMFE Group at x400 (5A) and x1000 (5B) Magnifications.

Key

"Red arrow" Islet cell

"Black arrow" Connective tissue

"Blue arrow" Interlobular septum

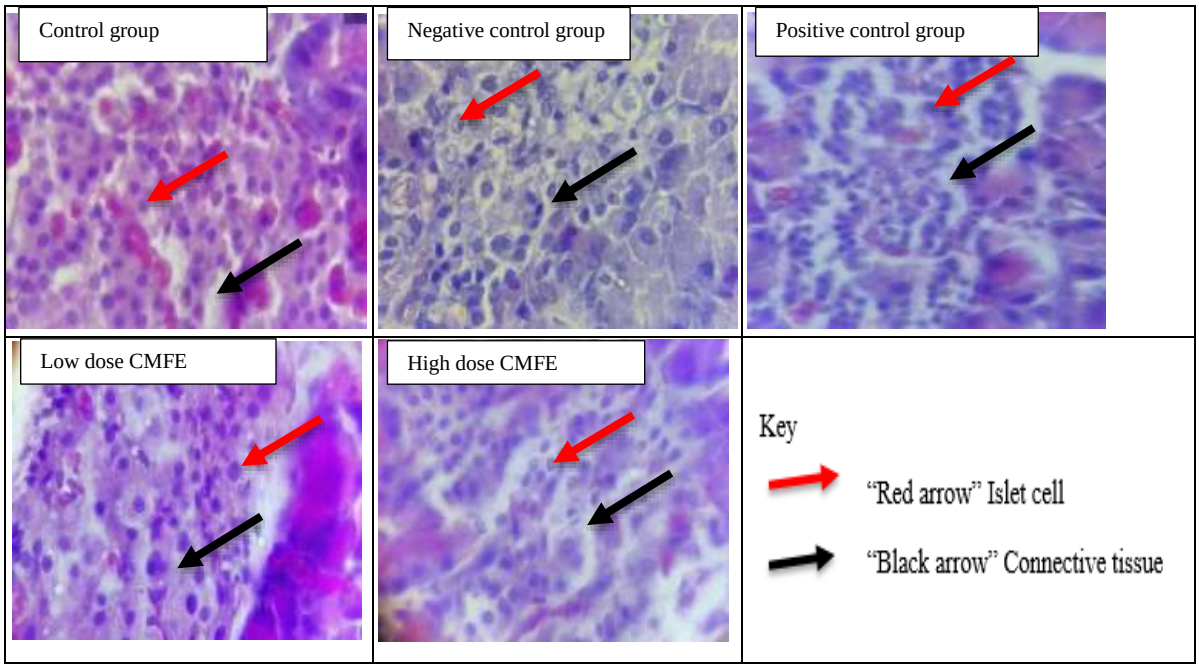

Figure 4.6: Photomicrographs (x1000 Magnification) Showing Variation of the Endocrine Pancreas Histomorphology in Different Treatment Groups

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Phytochemicals Present in Non-Bitter *Cucumis metuliferus* Fruit Extract

Phytochemical screening of non-bitter *Cucumis metuliferus* fruit extract revealed the presence of glycosides, alkaloids, saponins, and Tannins. The study findings agreed with those of Dhale (2011). However, the study did not establish the presence of flavonoids and phenols in *C. metuliferus,* as reported in studies done in South Africa (Maluleke *et al*., 2021) and Nigeria (Ezekaibeya *et al*., 2020). This could be due to soil types, treatment conditions, and the part of the plant used in the extraction (Maluleke, 2022).

5.1.2 Acute Oral Toxicity of Non-Bitter *Cucumis metuliferus* Fruit Extract

At a dose limit of 2000mg/kg body weight, CMFE exhibited no lethal toxicity, suggesting that the LD50 could be more than 2000mg/kg body weight. These findings are consistent with Wannang et al. (2008), who demonstrated that at a dose of 10 to 5000mg/kg body weight, the non-bitter CMFE did not cause death in Wistar albino rats. A study by Usman *et al.* (2018) also showed that at a dose limit of 5000mg/kg body weight, CMFE produced no toxicity signs or symptoms when administered orally to cockerels. This suggests that the extract, at doses of 2000mg/kg, is relatively safe. However, it was noted that at a dose of 2000 mg/kg body weight, physical activity (jumping, movement within the cage, making noise, and eating habits) increased. The findings agree with Jimam *et al*. (2012), who demonstrated that there was an increase in eating habits in Wistar rats when given *Cucumis metuliferus* fruit extract orally.

5.1.3 Effects of Non-Bitter *Cucumis metuliferus* Fruit Extract On Blood Sugar

On the effects of non-bitter CMFE on blood sugar, the study findings demonstrate that non-bitter CMFE downregulates blood sugar in type II diabetes mellitus in that treatment with a high-fat/fructose diet and streptozotocin significantly raised the fasting blood sugar (FBS) ($P < 0.001$) and ($P < 0.001$) on days 42 and 45, respectively, of the experiment. However, treatment with the non-bitter CMFE decreased the FBS to levels comparable to the control group on days 63 ($P = 0.90$) and 70 ($P = 0.163$) of the experiment. Similarly, the blood sugar in the oral glucose tolerance test significantly rose ($P < 0.001$) and ($P < 0.001$) on days 42 and 45, respectively, of the experiment, followed by a drop to levels comparable to the control group (Day 70 of the experiment) after treatment with the non-bitter CMFE. However, there was no significant difference in Hb AIC after treatment with a high-fat/fructose diet and streptozotocin, but treatment with pioglitazone significantly raised the Hb AIC ($P = 0.006$) on day 63 of the experiment. This demonstrates that the non-bitter CMFE is superior to PGZ in controlling blood sugar.

These findings are in tandem with a study conducted by Jimam *et al.* (2010), who demonstrated that CMFE possessed hypoglycemic effects in diabetic rats. Similarly, Kwaghe *et al*. (2015) described *Cucumis metuliferus* as one of the fruits that possess hypoglycemic properties. While previous studies focused on the short-term effects of CMFE on blood sugar, the current study findings demonstrate that *Cucumis metuliferus* fruit extract has long-term effects on the control of blood sugar, providing new insights into the use of *Cucumis metuliferus* fruit extract in the management of type II DM. These findings imply that the fruit extract can be used chronically to manage type II diabetes mellitus, preventing long-term complications associated with poor blood sugar control like coronary heart disease, which is a leading killer disease in the world (Al-Nozha *et al*., 2016). However, at a dose of 400 mg/kg CMFE (high dose), the rats had a significantly poor oral glucose tolerance at ($P = 0.002$) and ($P = 0.036$) on days 56 and 63, respectively, of the experiment, as opposed to what was observed at a dose of 200 mg/kg CMFE (low dose) on the same dates. A study by Jimam *et al*. (2012) demonstrated an increase in food intake by Wistar rats fed with CMFE, therefore suggesting that at a dose of 400 mg/kg

CMFE (a high dose), the rats consumed more food than at a dose of 200 mg/kg CMFE (a low dose), hence poor oral glucose tolerance. Consequently, this property of increased appetite can be utilised to the benefit of patients with poor food appetites.

5.1.4 Effects of Non-Bitter *Cucumis metuliferus* Fruit Extract on Liver Functions

Effects of Non-Bitter CMFE on Lipid Profile

Treatment with a high-fat/fructose diet significantly increased serum total cholesterol (TC) ($P < 0.001$), triglycerides (TGs) ($P = 0.019$), and low-density lipoproteins (LDL) ($P = 0.016$) on day 45 of the experiment. However, treatment with pioglitazone and non-bitter CMFE decreased these parameters to levels comparable to the control group on days 63 and 70 of the experiment. On the contrary, treatment with a high-fat/fructose diet significantly decreased ($P = 0.004$) serum high-density lipoproteins (HDL) on day 45 of the experiment, which was followed by a significant rise ($P = 0.001$) after treatment with non-bitter CMFE, although this was not observed in the PGZ-treated group on day 70 of the experiment, suggesting that non-bitter CMFE is superior to PGZ in the regulation of serum lipids.

Dyslipidemia observed on day 45 of the experiment is consistent with studies by Ahmed *et al*. (1998) and DiNicolantonio & O'Keefe (2018) that demonstrated prolonged consumption of a high-fat diet causes dyslipidemia in humans. The findings also agree with Namekawa *et al*. (2017), who demonstrated that when Wistar rats were fed a high-cholesterol and fructose diet for four (4) weeks, they exhibited signs and symptoms of obesity and hyperglycemia. Udomkasemsab & Prangthip (2019) also demonstrated that a high-fat diet causes dyslipidemia.

While type II diabetes mellitus is associated with an increased risk of developing dyslipidemia, diabetic patients require statin therapy (Daya *et al.,* 2017). This study demonstrates that the non-bitter CMFE may be used to treat diabetic-induced dyslipidemia, thereby reducing the pill burden. Consequently, this lowers the risk of

developing cardiovascular complications (Hedayatnia *et al*., 2020). Although the studies reviewed failed to address the extract's hypolipidemic properties, these findings provide new insight into the use of CMFE as a remedy in the management of diabetic and dietary-induced dyslipidemia.

Effects of Non-Bitter *Cucumis metuliferus* Fruit Extract on Liver Enzymes

Treatment with streptozotocin significantly increased serum alanine aminotransferase (ALT) ($P < 0.001$), aspartate aminotransferase (AST) ($P < 0.001$), and gamma-glutamyl transferase (GGT) ($P = 0.002$) on day 45 of the experiment. However, treatment with non-bitter CMFE decreased these liver enzymes to levels comparable to the control group on day 70 of the experiment.

The rise in serum ALT, AST, and GGT on day 45 of the experiment is consistent with the study findings described by Omonkhua *et al.* (2014), which demonstrated hepatocellular damage following treatment with streptozotocin to induce diabetes mellitus in rats. Sprinzl *et al.* (2013) also demonstrated hepatic injury after treatment with streptozotocin in rats. However, a decrease in these liver enzymes to levels comparable to the control group after treatment demonstrates that non-bitter CMFE has hepato-restorative properties, a property that can be attributed to non-bitter CMFE's direct effect on the liver or its indirect effect through control of blood sugar. These findings agree with Anyanwu *et al*. (2014), who concluded that alkaloids present in non-bitter *Cucumis metuliferus* are linked to the fruit's hepato-restorative effects. In a review article, Vishwakarma *et al.* (2017) also concluded that the *Cucumis metuliferus* plant possesses hepato-restorative properties. This is an added advantage when the non-bitter CMFE is used in the management of diabetes mellitus since type 2 diabetes has been linked to serious liver damage due to the disease's characteristic insulin resistance and oxidative stress (Mohamed *et al.*, 2016b; Tolman *et al.*, 2007).

Effects of Non-Bitter *Cucumis metuliferus* Fruit Extract on Serum Proteins

Treatment with non-bitter CMFE significantly increased serum total proteins (TP) (P =0.002) and (P =0.023) on days 63 and 70, respectively, of the experiment. Similarly, there was a significant rise in serum albumin (ALB) (P =0.024), (P <0.001), and (P = 0.001) on days 56, 63, and 70, respectively, of the experiment. However, this was observed in the group treated that received high-dose CMFE but not in the low-dose CMFE group or positive control group, an indication that the findings were dose-dependent and CMFE is superior to PGZ.

Although previous studies failed to address these properties, a study by Bae *et al.* (2013) suggested that increased serum albumin levels are associated with hyperinsulinemia. Chen *et al.* (2016) also demonstrated that albumin secretion was reduced significantly in diabetic type 1 mice, with a possible explanation being the absence of insulin. Wanke & Wong (1991) concluded that insulin promotes transcription of the albumin gene, resulting in increased production of albumin. In their study, Devaprashanth *et al*. (2021) demonstrated that patients who had type I diabetes mellitus also had hypoalbuminemia.

These findings strongly suggest that non-bitter CMFE stimulates beta cells, increasing insulin hormone production and hence increasing the synthesis of albumin and overall total protein.

Effects of *Cucumis metuliferus* Fruit Extract on Serum Bilirubin

Treatment with streptozotocin significantly decreased (P =0.031) serum total bilirubin on day 45 of the experiment. However, treatment with PGZ and non-bitter CMFE decreased serum total bilirubin to levels comparable to the control group on days 56, 63, and 70 of the experiment. The decrease in serum total bilirubin observed on day 45 of the experiment could be attributed to the development of type II DM following treatment with streptozotocin.

These findings corroborate those of Erkus *et al.* (2018), who established that individuals with type 2 diabetes mellitus who had serum bilirubin levels within normal ranges had good blood sugar control.

5.1.5 Effects of *Cucumis metuliferus* Fruit Extract on Total Body Weight

Treatment with a high-fat/fructose diet significantly increased ($P > 0.033$) mean total body weight on day 42 of the experiment. However, treatment with streptozotocin significantly decreased mean total body weight on days 56 ($P < 0.001$), 63 ($P < 0.001$), and 70 ($P < 0.001$) of the experiment.

The increase in total body weight noted in all treatment groups on day 42 of the experiment could be attributed to treatment with a high-fat and fructose diet for 6 weeks before treatment with STZ to induce type II DM, findings that are consistent with Coelho *et al*. (2011), who concluded that consumption of a high-fat diet leads to an increase in total body weight. Ozougwu (2013) also established that prolonged consumption of a high-fat diet leads to an increase in body weight, a risk factor for the development of type II DM.

The decrease in total body weight observed after treatment with STZ to induce type II diabetes on days 56, 63, and 70 of the experiment could be linked to the development of type II DM. These findings are in tandem with those of Song *et al*. (2019), who demonstrated that diabetes mellitus disease progression leads to overall body weight loss. The findings suggest that CMFE did not affect total body weight in type II DM.

5.1.6 Effects of *Cucumis metuliferus* Fruit Extract on the Histomorphology of the Islet of Langerhans

There was a uniform distribution of the cells in the Islet of Langerhans, with a homogenous distribution of connective tissue matrix and few blood vessels in the control group. A marked reduction of cell density in the Islet of Langerhans was observed in the negative control group, which could be linked to beta cell death following STZ injection. Cells in the Islet of Langerhans had pyknotic nuclei, which were also less basophilic when

compared to the control group. The study findings agree with those of Wilson and Islam (2012), who demonstrated beta cell dysfunction in type 2 diabetic rats. A study by Widyawati *et al*. (2021) also demonstrated how STZ destroys the structure of the pancreatic Islet of Langerhans in rats. The positive control group demonstrated a sparse population of cells in the islet of Langerhans, but they were basophilic when compared to the control group, an indication that pioglitazone possesses some restorative properties in the islet of Langerhans. Treatment with low-dose CMFE demonstrated cell distribution comparable to that of the control group but with a lesser cell population than the positive control. The treatment group that received high doses of CMFE had more basophilic nuclei when compared to the control, negative control, and positive control groups. This is an indication that CMFE possesses dose-dependent restorative properties. It is also a possibility that CMFE exerts some of its mechanism of action at the Islet of Langerhans level, probably by stimulating beta cells to produce more insulin in response to the pathophysiology of type 2 diabetes mellitus.

In general, this study demonstrates that the CMFE safety profile is relatively high and that the fruit possesses hypoglycemic, hypolipidemic, and hepato-restorative properties in type II diabetes mellitus. The likely mechanism of action in the control of blood sugar is the stimulation of insulin-producing beta cells in the Islet of Langerhans.

Limitations of the study

During the experiment, some rats died due to complications of type II diabetes mellitus. However, at the design stage and in anticipation, each group had an extra fifteen per cent increase in the number of rats, and replacement was done accordingly. Although the experiment was completed in April 2020, the COVID-19 pandemic led to the closure of all learning institutions, including JKUAT, Kenya, and this led to the postponement of histological analysis of the pancreas since the histology lab in JKUAT was inaccessible. However, this did not affect the results since the tissues were preserved appropriately.

5.2 Conclusion

This study concludes that;

1. The non-bitter CMFE contains glycosides, alkaloids, saponins, and tannins.
2. The LD_{50} for non-bitter CMFE in rats is higher than the highest dose administered, i.e., 2,000 mg/kg body weight. However, at a dose of 2000 mg/kg body weight, increased physical activity (jumping, movement within the cage, making noise) and eating habits observed suggest that the extract may be a psychostimulant and an appetizer.
3. The non-bitter CMFE possesses both short-term and long-term hypoglycemic properties, as evidenced by its capacity to lower FBS and OGTT and the fact that glycated haemoglobin did not change in type II DM following treatment.
4. The non-bitter CMFE possesses hepato-restorative and hypolipidemic properties, as evidenced by the extract's ability to decrease elevated liver enzymes, regulate bilirubin levels, and correct nutritional and type II diabetes-induced dyslipidemias.
5. The non-bitter CMFE increases the metabolic activity of insulin-producing beta cells in the islets of Langerhans.

5.3 Recommendations

This study recommends;

1. Further studies to establish the chronic toxicity profile of non-bitter CMFE
2. Use of non-bitter CMFE as an adjunct treatment remedy in the management of type II diabetes mellitus
3. Using non-bitter *Cucumis metuliferus* fruit extract in managing diabetic and nutritionally induced dyslipidemias.
4. Further studies to confirm the extract's mechanism of action in the control of blood sugar.

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APPENDICES

Appendix I: Animal Ethics Review Committee Approval Letter

JOMO KENYATTA UNIVERSITY
OF
AGRICULTURE AND TECHNOLOGY
P. O. Box 62000-00200 Nairobi, Kenya Tel 0675870225 OR Extn 3209
Animal Ethics Review Committee

January 21st, 2019 **REF: JKU/2/4/896C**

Dennis Mwangi Muriuki,
HSM 302-4019/2016
Department of Medical Physiology.

Dear Mr. Muriuki,

RE: EFFECTS OF *CUCUMIS METULIFERUS* (KIWANO) FRUIT EXTRACT ON THE METABOLIC PROFILES OF STREPTOZOTOCIN-INDUCED DIABETIC ALBINO RATS

The JKUAT Animal Ethics Review Committee has reviewed your responses to issues raised regarding your application to conduct the above mentioned study with you as the Principal Investigator.

The is to inform you that the AERC has approved your protocol. The approval period is from January 21st 2019 to January 21st 2020 and is subject to compliance with the following requirements:

a) Only approved documents (informed consent, study instruments, study protocol, etc.) will be used.
b) All changes (amendments, deviations, violations, etc.) must be submitted for review and approval by the JKUAT AERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the IERC immediately.
d) Any changes, anticipated or otherwise that may increase the risks to or affect the welfare of study participants and others or affect the integrity of the study must be reported immediately.
e) Should you require an extension of the approval period, kindly submit a request for extension 60 days prior to the expiry of the current approval period and attach supporting documentation.
f) Clearance for export of data or specimens must be obtained from the JKUAT AERC as well as the relevant government agencies for each consignment for export.
g) The IERC requires a copy of the final report for record to reduce chances for duplication of similar studies.

Should you require clarification, kindly contact the JKUAT AERC Secretariat.

Yours Sincerely,

Dr. Patrick Mbindyo
SECRETARY, AERC

J.K.U.A.T
DIRECTOR,
RESEARCH DEPAR... (RPE)
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NAIRO...

Setting Trends in Higher Education, Research and Innovation

Appendix II: Publication

ORIGINAL RESEARCH ARTICLE

The effects of non-bitter *Cucumis metulijerus* fruit extract on blood sugar of high-fat/fructose diet and streptozotocin-induced type II diabetic Wistar Albino rats

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ABSTRACT

Globally, diabetes mellitus (DM) remains one of the leading debilitating non-communicable diseases, with its prevalence projected to increase from 8.8% in 2015 to 10.0% in 2030. The management of diabetes mellitus remains a major challenge, and the number of diabetes-related deaths is projected to rise from 3.1 million in 2015 to 4.2 million in 2030. This continues to be a public health concern, particularly in developing countries where the majority of people are poor and predominantly live in rural areas, facing challenges in accessing healthcare services. However, the non-bitter *Cucumis metulijerus* fruit is being used for managing type 2 diabetes mellitus by some communities in Kenya, although its therapeutic benefits have not been adequately studied and proven. The study aimed to determine the effects of non-bitter *Cucumis metulijerus* fruit extract on blood sugar in high-fat/fructose diet and streptozotocin-induced type II diabetic Wistar albino rats. This study adopted an experimental laboratory-based design. A sample size of 64 male Wistar albino rats, aged 5 weeks and weighing between 90 and 130 grams, was randomly assigned to two major study groups: the control group and the experimental group. The experimental group received a high-fat/fructose diet plus streptozotocin (STZ) injection to induce diabetes mellitus, whereas the control group received a standard rodent pellet diet plus 0.9% normal saline injection. The experimental group was further divided into a positive control group treated with pioglitazone (the standard drug) at a dose of 20 mg/kg body weight, a low-dose CMFE group at 200 mg/kg body weight, and a high-dose CMFE group at 400 mg/kg body weight. Fasting blood sugar (FBS), oral glucose tolerance test (OGTT), and haemoglobin A_{1c} (Hb A_{1c}) tests were used as indicators, and the results were compared between the groups. The study findings revealed a significant statistical rise ($P < 0.001$) of FBS in the treatment group after induction of type II DM, followed by a decline to pre-induction levels after treatment. Similarly, there was a statistically significant increase ($P < 0.001$) of the OGTT after induction of type II DM, with the OGTT declining to pre-induction

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