ORIGINAL RESEARCH ARTICLE

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

Muriuki Dennis Mwangi[1], Kamau David Muchina[1], Kweri Joseph Kariuki[2], Karanja Simon[3], Thuo Reuben[4]

[1]*Department of Medical Physiology, Jomo Kenyatta University of Agriculture and Technology, Kenya*
[2]*Department of Human Anatomy, Jomo Kenyatta University of Agriculture and Technology, Kenya*
[3]*Department of Environmental Health and Disease Control, Jomo Kenyatta University of Agriculture and Technology, Kenya*
[4]*Department of Surgery, Jomo Kenyatta University of Agriculture and Technology, Kenya*

Corresponding author: ***muriukidennis420@gmail.com***

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 metuliferus* 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 metuliferus* 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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levels following treatment (P =0.106). The findings on both the FBS and OGTT tests indicate that treatment with CMFE controlled the blood sugar. Consequently, there was no significant difference (P =0.712) in Hb A_{1c} test results between the control group and the treatment group at the end of the experiment, indicating that treatment with CMFE had a long-term control effect on blood sugar. This study concludes that the non-bitter CMFE possesses both short-term and long-term hypoglycemic properties in type II diabetes mellitus.

Key Word: Streptozotocin; *Cucumis metuliferus*; herbal remedy; fasting blood sugar; oral glucose tolerance test; haemoglobin A1c.

1.0 Introduction

Worldwide, the control and management of type II diabetes mellitus continue to be a challenge because of the associated long-term cost, which is not only expensive but a financial burden to the patients throughout their lives (Ozougwu, 2013). According to Ly *et al*. (2022), Kenya's poverty level stood at 36.1% in 2015, with enrollment in universal health insurance reaching 19% in 2016. This has not only negatively impacted health promotion, particularly for the rural population where access to affordable and quality health care services has remained a challenge, but also for diabetic patients who instead turn to traditional healers when access to specialist health practitioners is restricted or the cost of conventional medicine is not affordable (Lambert *et al*., 2011). As a consequence, many people have turned to herbal medicine, among them Cucumis metuliferus fruit, in the management of type II diabetes mellitus. However, the fruit's therapeutic claim as a complement to inherent insulin or oral hypoglycemic agents in the management of type II diabetes mellitus is not well elucidated, hence the basis of this study.

2.0 Material and Methods

2.1 Study area

This study was conducted in the Small Animal Facility for Research and Innovation (SAFARI) at Jomo Kenyatta University of Agriculture and Technology (JKUAT), Kenya, between December 2019 and April 2020.

2.2 Study design

A laboratory-based experimental study design was adopted.

2.3 Type of animal model used

According to Sood *et 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.

2.3.1 Acquisition of Wistar Albino rats

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

2.3.2 Selection of rats

Five (5) week-old male Wistar albino rats weighing between 90 and 130 grams were selected

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and included in this study.

2.3.3 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 they had free access to approved rodent pellet food and clean water according to the experimental protocol.

2.4 Sample size calculation

The sample size was arrived at using the "resource equation method" (Charan and Biswas, 2013; Mwangi *et al.*, 2023).

E = total number of animals x Total number of groups
Total number of animals: number of animals per group x number of groups
E = (number of groups x number of animals per group) – number of groups
E is the degree of freedom of the 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, and the data collected in this study was considered scientifically adequate,

2.5 Grouping of rats

The male Wistar Albino rats that constituted a sample size of 64 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/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 that was 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. All the groups had subgroups "a", "b", c," and "d" each to cater for serial sampling on the 45th, 56th, 63rd, and 70th days of the experiment.

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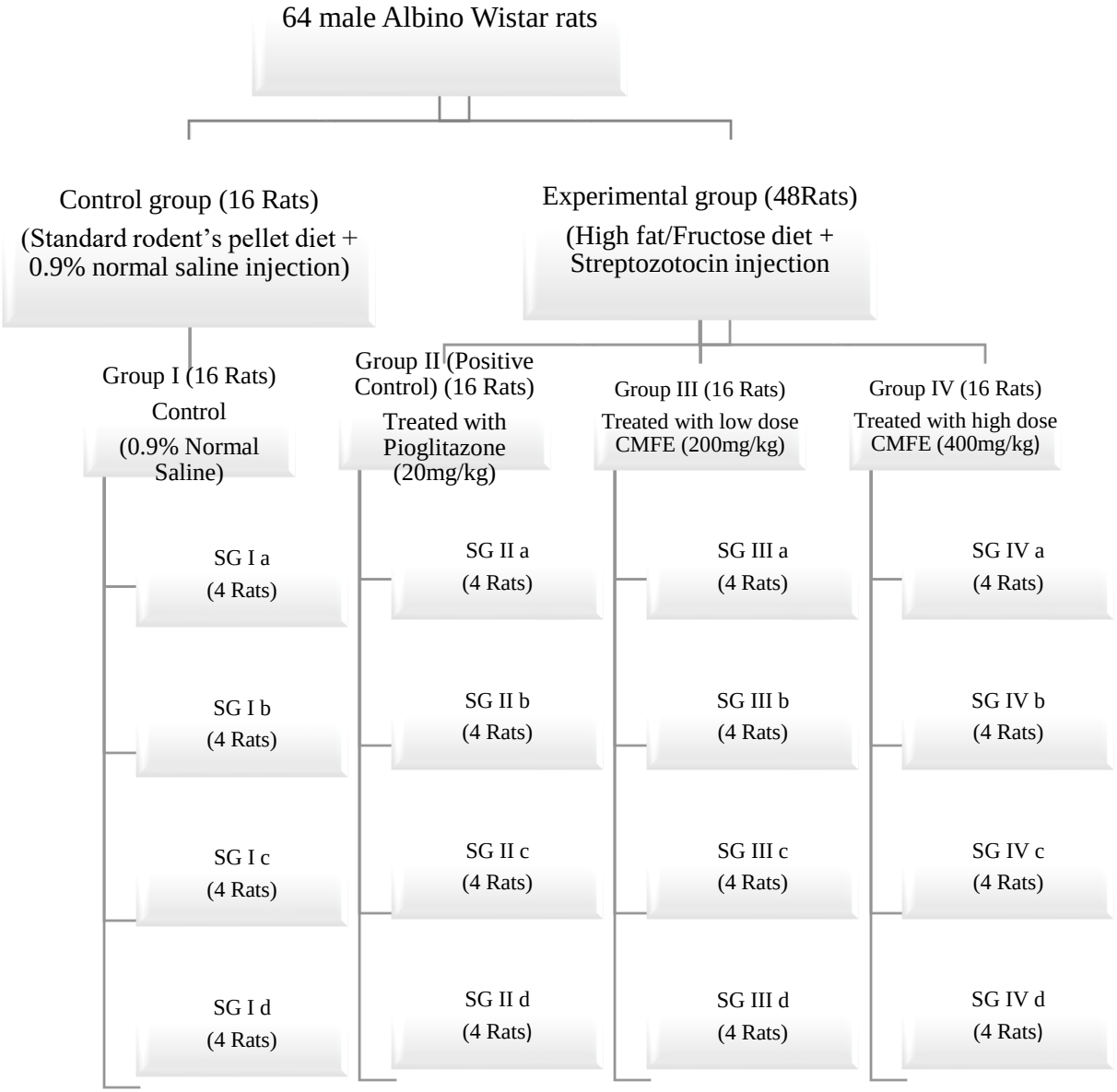

Figure 3.1 Grouping of animals and treatment

2.6 Harvesting of non-bitter *Cucumis metuliferus* fruit

The non-bitter form of *Cucumis metuliferus* fruits were 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.

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2.6.1 Extraction of non-bitter *Cucumis metuliferus* crude fruit extracts

The extraction of the fruit was done using the maceration technique as described by Zhang *et al*. (2018). Whole fruits were washed off of dirt and any foreign bodies using clean water, 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 pressed to recover as much of the occluded solution as possible. The liquid was then filtered to remove particles using muslin cloth, followed by filter paper. Ethanol was then evaporated using a rotary evaporator, and freeze drying followed to retain non-bitter *Cucumis metuliferus* fruit extract paste. The paste was stored at 4 degrees Celsius until use.

2.7 Preparation of a high-fat and fructose diet for the rats

On the preparation of a high-fat diet, this study adopted a protocol developed by Animales, Nutricional, and Tipo (2014); Mwangi et al. (2023), which states that if 30%–50% 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 grammes of frymate (which provided 900 kcal per 100-gramme serving) were mixed with 100 grammes of standard rat pellet, 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.

On the preparation of a fructose diet, this study adopted a protocol developed by Wilson and 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 container containing 10 grams of fructose while stirring to make a 100-mL solution. The experimental rats had access to fructose solutions *ad libitum* throughout the experiment.

2.8 Feeding of the rats

The control group was fed with a standard rats' pellet composed of carbohydrates (65.3%), crude protein (18.1%), crude fibre (7%), fat (8%), calcium (0.8%), and phosphorous (0.8%), plus clean water. The experimental group was fed a high-fat diet composed of carbohydrates (55.5%), fat (21.8%), crude protein (15.4%), crude fibre (6%), calcium (0.7%), phosphorous (0.7%), and fructose water. All rats received food and water *ad libitum*.

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2.9 Confirmation of non-diabetic status before treatment with streptozotocin

Fasting blood sugar (FBS) test and oral glucose tolerance test (OGTT) were carried out to confirm that there was no diabetic case before treatment with streptozotocin to induce diabetes mellitus. The rats fasted for 6–8 hours during the morning hours. After fasting, a drop of whole blood was collected through a tail prick to test for FBS, followed by OGTT. The rat that had an FBS of <7 mmol/L and an OGTT of <11.1 mmol/L was included in the study (Patel & Macerollo, 2010).

2.10 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.

2.11 Treatment with streptozotocin to induce diabetes mellitus

Treatment with STZ was done on day 42 of the experiment. Before treatment with STZ, the rats fasted for 6–8 hours in the morning. After fasting, the experimental group received a single intra-peritoneal injection of freshly prepared streptozotocin (40 mg/kg body weight) to induce type II DM. The control group received a single intraperitoneal injection of 1 ml of 0.9% normal saline..

2.12 Confirmation of diabetes mellitus (DM)

Testing was done on day 45 of the experiment to confirm the presence of DM. The rats were fasted for 6–8 hours in the morning hours, and after fasting, a drop of whole blood was collected through a tail prick to test for FBS, followed by OGTT. The rat that had an FBS of >7 mmol/L and an OGTT of >11.1 mmol/L was considered diabetic and was included in the experimental group but excluded from the control group.

2.13 Determination of non-bitter *cucumis metuliferus* fruit extract dose

According to Jimam *et al*. (2012), on the histopathological effects of non-bitter *Cucumis metuliferus* fruit extract obtained through soxhlet ethanol extraction in albino rats, at a dose of 1000 mg/kg, there was evidence of tissue necrosis and degeneration of the liver, while the kidneys showed some features of renal epithelial cell damage. However, at a dosage of 500 mg/kg body weight, there was no significant alteration of liver or renal tissues as compared to the control. The tissues of the spleen and pancreas of rats treated with both doses of 500 mg/kg and 1000 mg/kg of *Cucumis metuliferus* fruit extract were normal when compared with the control group.

Since there was no human data that could be used to calculate the dosage of non-bitter *Cucumis metuliferus* crude extract, with reference to the above study and the acute oral toxicity study of CMFE conducted prior to the current study, a dose of 400 mg/kg body weight was adopted as the maximum therapeutic dose (high dose) and 200 mg/kg body weight as the minimum therapeutic dose (low dose) of CMFE. The low dose was calculated using the natural

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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%

Where e^ represents the log

Highest dose=400
e^ of 90%=1-0.9=0.1=0.1054
Least dose = 400 x e^ (-0.1054) =Approx. 200
Highest dose=400

2.13.1 Preparation and administration of the non-bitter *Cucumis metuliferus* fruit extract solution

The non-bitter CMFE paste was reconstituted using 5% DMSO to make a solution of 100mg/ml of 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 of the experiment.

2.14 Determination of pioglitazone dose

A study on the evaluation of the biochemical toxicity properties of pioglitazone in Albino Wistar rats concluded that at doses of 15mg/kg to 45 mg/kg body weight, the drug exhibited no biochemical toxicological effects (Ogunlana *et al*., 2017). This study adopted a dose of 20mg/kg of pioglitazone as described by (Chege *et al*., 2019).

2.14.1 Preparation and administration of pioglitazone (PGZ) solution

Pioglitazone solution was prepared by dissolving a 30mg tablet in 5% DMSO to make 6 ml of the mixture, giving a concentration of 5 mg per 1 ml of the solution. The drug was administered at a dose of 20 mg/kg body weight via oral gavage. Treatment commenced immediately after confirmation of diabetes mellitus on day 45 and continued until day 70 of the experiment.

2.15 Procedure for humane killing of the rats and collecting tissue specimens

The humane killing of the rats and the collection of tissue specimens were carried out in the SAFARI procedure room. First, the rats were fasted for 6–8 hours before being sacrificed. The rat was then 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. The anaesthesia was allowed to take effect for 3 to 5 minutes, after which 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 its hypodermic needle, approximately 5 ml of blood was collected from

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the heart through direct intra-cardiac puncture and immediately emptied into the appropriate labelled collecting test tubes. The samples were stored in a refrigerator at temperatures between 2 and 8 degrees Celsius, awaiting transportation to a 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, the desired fixative (10% formalin solution) was introduced slowly into the circulation of the rat. The firmness of the tail was checked as a sign of effective fixation.
The perfusion needle was then disconnected and removed from the heart. The pancreas was then carefully excised and immersed in a clearly labelled container with fresh fixative to continue fixation for 12 hours.

2.16 Blood sugar tests

2.16.1 Testing procedure for fasting blood sugar (FBS) and oral glucose tolerance test (OGTT)

FBS and 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 manufactured by Roche Diabetes Care Inc. After fasting the rats for 6–8 hours, the rat to be tested was put in a rodent restrainer. When the rat 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 dosage of 2g/kg body weight, and using the same procedure, 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 and an average OGTT of more than 11.1 mmol/L were considered diabetic (Patel & Macerollo, 2010).

2.16.2 Testing procedure for glycated haemoglobin (Hb A_{1c})

HbA1c was tested on days 45, 56, 63, and 70 of the experiment, and the test was carried out using the Clover A_{1c} Self HbA1c analyzer manufactured by Infopia Co., Ltd. After fasting the rats for 6–8 hours, the rat to be tested was put in a rodent restrainer. When the rat was safely secured, its tail was cleaned using an alcohol-benzocaine pad (benzocaine 6%, isopropyl alcohol

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70% v/v) and allowed to air dry for approximately 30 seconds. Using a lancet, the tail was pricked, and a drop of whole blood was allowed out. 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 haemoglobin 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 haemoglobin from the blood sample so that glycated haemoglobin could be photometrically measured. The ratio of glycated haemoglobin to total haemoglobin 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 Hb A_{1c} ranging between 4% and 6.4% (Patel & Macerollo, 2010).

2.17 Data analysis

Raw data was keyed into a Microsoft Excel Spreadsheet and then transferred to Statistical Package for Social Sciences (SPSS) version 25 software for analysis. Descriptive statistics such as mean, median, frequencies, and standard deviation were generated. A comparison of multiple means was done using ANOVA. The Tukey statistical test was used for post-hoc statistical analysis. The analysis was done at a 95% level of confidence (P 0.05) and the data was presented in tables. Qualitative data was analysed and described by comparing different observations. Results were disseminated to the university and research community in the form of thesis and seminars.

3.0 Results

3.1 Effects of non-bitter C*ucumis metuliferus* fruit extract (CMFE) on fasting blood sugar (FBS)

There was a significant statistical difference when FBS test results were compared between the control group and the experimental group on days 42 (P 0.001), 45 (P 0.001), and 56 (P = 0.030), but no 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 group treated with 20 mg/kg PGZ (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. The rise of FBS, although not above 7 mmol/L, could be explained by treatment with a high-fat/fructose diet for 6 weeks before treatment with STZ to induce type II DM on day 42 of the experiment. There was a significant rise (P <0.001) in FBS in the group treated with 20 mg/kg PGZ (13.96±2.790 test vs. 3.37±1.011 control), 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. The rise in FBS observed on day 45 confirmed the development of hyperglycemia after treatment with STZ. A significantly high (P = 0.030) FBS, although not above 7 mmol/L, was also noted in the group treated with 20 mg/kg PGZ (6.53±1.527 test vs. 2.96±0.551 control) on day 56 of the experiment. These results on day 56 of the experiment indicate that treatment with CMFE had better results in controlling FBS in

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comparison to treatment with PGZ. However, there was no significant difference observed on days 63 (P = 0.090) and 70 (P = 0.163) of the experiment confirming that treatment with CMFE was comparable to treatment with PGZ (the standard drug in this study) in the regulation of FBS. (Table 3.1).

Table 3.1: A table showing results of the effects of non-bitter cucumis metuliferus fruit extract on fasting blood sugar (Expressed in mmol/L)

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

Notes: Using one-way ANOVA and Tukey test on post-hoc. * indicates significance (p<0.05).
Key: FBS=Fasting Blood Glucose

3.2 Effects of non-bitter C*ucumis metuliferus* fruit extract on oral glucose tolerance test (OGTT)

There was a significant statistical difference when OGTT test results were compared between the control group and treatment groups on days 42 (P < 0.001), 45 (P <0.001), 56 (P =0.002), and 63 (P = 0.036), but no significant statistical difference (P =0.106) on day 70 of the experiment. Post hoc statistical analysis using the Tukey test revealed a significantly high (P <0.001) OGTT in all treatment groups on day 42 of the experiment. Although less than 11.1 mmol/L, the rise could be attributed to treatment with a high-fat/fructose diet for 6 weeks prior to treatment with STZ to induce type II DM, hence insulin resistance. There was a significant statistical increase (P <0.001) in OGTT in all treatment groups on day 45. The rise observed on day 45, which was above 11.1 mmol/L, 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 20 mg/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. Although below 11.1 mmol/L, the test results on day 56 of the experiment indicates poor OGTT in the positive control group and in the group treated with high-dose CMFE. There was 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 OGTT. However, sustained OGTT test results of less than 11.1 mmol/L following treatment with CMFE confirm that treatment with the non-bitter CMFE controlled the blood sugar in type II DM. (Table 3.2).

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Table 3.2: A table showing results of the effects of non-bitter cucumis metuliferus fruit extract on oral glucose tolerance test (Expressed in mmol/L)

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	8.640±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

Notes: Using one-way ANOVA and Tukey test on post-hoc. * indicates significance ($p<0.05$).
Key: OGTT=Oral Glucose Tolerance Test

3.3 Effects of non-bitter *cucumis metuliferus* fruit extract on long-term control of blood sugar using Hb A_{1c} as an indicator

There was no significant statistical difference (P = 0.224) when Hb A_{1c} test results were compared between the control group and the experimental group 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 Hb A_{1c}. However, there was a significant statistical difference (P = 0.006) in Hb A_{1c} on day 56 of the experiment, but no 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) in 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. These results demonstrate that CMFE chronically controlled the blood sugar, as evidenced by sustained mean Hb A_{1C} levels of less than 6.5% throughout the experiment (Table 3.3).

Table 3.3: A table showing results of the effects of cucumis metuliferus fruit extract on long-term control of blood sugar using HbA1c as an indicator (Expressed in %)

Test day	Control	Positive control PGZ (20mg/kg)	Low dose CMFE (200mg/kg)	High dose CMFE (400mg/kg)	F	P-value
45th day	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

Notes: Using one-way ANOVA and Tukey test on post-hoc. * indicates significance ($p<0.05$).
Key: Hb A_{1c} = Haemoglobin A_{1c}

4.0 Discussion

4.1 Effects of non-bitter *cucumis metuliferus* fruit extract (CMFE) on blood sugar

On the effects of non-bitter CMFE on blood sugar, the study findings demonstrate that non-bitter CMFE controls blood sugar in type II diabetes mellitus.

Following induction of DM on day 42, by day 45, FBS in the experimental group rose significantly (P < 0.001) in comparison to the control group. However, after treatment with CMFE, the FBS levels dropped to levels similar to those of the control group on days 63 (P = 0.90) and 70 (P =

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0.163). Similarly, oral glucose tolerance test levels were significantly increased (P <0.001) before treatment with CMFE on day 45 of the experiment, after which there was no significant difference (P =0.106) between the control group and the treatment groups on day 70. Furthermore, there was no significant difference in HB AIC between the control group and the treatment groups on days 45 (P = 0.224), 63 (P = 0.079), and 70 (P = 0.712) of the experiment. Although there is limited knowledge of the effects of CMFE on human 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 a*l. (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 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.

5.0 Conclusion

This study concludes that the non-bitter CMFE has hypoglycemic properties in type II diabetes mellitus because blood sugar levels returned to pre-induction levels when oral glucose tolerance test (OGTT) and fasting blood sugar (FBS) tests were used to measure blood sugar levels. Similarly, during the experiment, the blood sugar levels in the treatment groups were comparable to those in the control group when the haemoglobin A_{1c} test was used to monitor chronic blood sugar control.

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None

6.2 Ethical approval

Ethical approval (REF: JKU/2/4/896C) was sought from the JKUAT Animal Ethics Review Committee. Ethical conduct was observed at all times during the entire period of the study.

6.3 Conflict of interest

The authors declare no conflict of interest

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