EVALUATION OF THE ANTIDIABETOGENIC EFFECTS OF THE FREEZE-DRIED EXTRACTS OF *Rotheca myricoides* ON TYPE 2 DIABETES IN AN ANIMAL MODEL

BY

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REG. NO. H56/89241/2016

A RESEARCH THESIS SUBMITTED IN PARTIAL FULFILLMENT FOR THE AWARD OF A DEGREE IN MASTER OF SCIENCE IN MEDICAL PHYSIOLOGY OF THE UNIVERSITY OF NAIROBI.

APRIL, 2019

DECLARATION

I hereby declare that this thesis is my original work and to the best of my knowledge has not been presented elsewhere for approval and for the award of a degree, diploma or certificate.

I further declare that all material cited in this thesis that is not my own work has been duly referenced.

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ACKNOWLEDGEMENTS

I raise my heart in gratitude to God almighty for all the blessings. He has been the guiding force behind my efforts. I wish to express my sincere thanks and appreciation especially to the following, My supervisors, Dr Peter Waweru Mwangi and Dr Frederick Bukachi for their scholarly guidance, availability at all times, constant encouragement and keen interest during the course of this study. Prof. Owino Okong'o, Prof. Nilesh Patel for the teaching in various areas of medical physiology and research. All members of the Department of Medical Physiology, for providing a very friendly working environment, especially to the department administrators i.e. Caroline Makandi and Elizabeth Juma. The technical staff, Mr. David Wafula, Mr Horo Mwaura, Mr. Jackson Mugweru and Mr. Thomas Arani, for their encouragement, availability at all time and all the support given during this study. Dr Zephaniah Birech and Mr. Moses Juma for their assistance with Raman Spectroscopy. My colleagues, Prabjot Sehmi and Nelly Nyaga for the cheerful atmosphere and inputs they provided throughout the study. My Parents Mr. Samson Chege & Mrs. Jane Chege for their warm care and support, my beautiful wife Christine Mwangi, my handsome boys Ethan Chege and Ellis Githae for always staying by my side.

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LIST OF ABBREVIATIONS AND ACRONYMS

RMFE	*Rotheca myricoides* freeze dried extract
T2D-	Type 2 diabetes
FBG-	Fasting blood glucose
HFD-	High-fat diet
MODY	Maturity-onset diabetes of the young
DM	Diabetes mellitus
STZ	Streptozocin
C/EBP	CCAAT-enhancer-binding protein
GLUT 4	Glucose transporter type 4
DAG	Diacylglycerol
PKC	Protein kinase C
HOMA-IR	Homeostatic model of assessment of insulin resistance
AUC'S	Area under the curve
OGTT	Oral glucose tolerance test
MSG	Oral glucose tolerance test
OD	Optical density

ABSTRACT

Type 2 diabetes is a complex metabolic disorder essentially characterized by alterations in lipid metabolism, insulin resistance and pancreatic β-cell dysfunction. *Rotheca myricoides* (Hochst.) Steane & Mabb is a plant frequently used in traditional African medicine in the management of diabetes. Type 2 diabetes accounts for 90% of diabetes with obesity being the most common risk factor for the development of T2D. Current treatment modalities do not cure or reverse the progression of the disease as end organ damage still develops despite adequate glycaemic control, underscoring the need for newer more efficacious drugs for the management of T2D. This study evaluated the antidiabetogenesis effects of freeze-dried extracts of *Rotheca myricoides* (Hochst.) Steane & Mabb in a diet and low dose streptozocin type 2 diabetes animal models**.**

Type 2 diabetes was induced by dietary manipulation for 56 days and intraperitoneal administration of streptozocin (30 mg/kg). The extracts and pioglitazone were administered throughout the study period by daily oral gavage. The study was conducted in two phases. In the first phase (efficacy experiment), forty (40) freshly-weaned *Sprague Dawley rats* were randomly assigned into the negative control (high fat/ high fructose diet), low dose test (50mg/kg RMFE), high dose test (100mg/kg RMFE) and positive control (Pioglitazone, 20mg/kg) groups. In the second phase (mechanism of action experiment), twenty-four rats (24) were randomly assigned to the negative control (normal saline), positive control (50mg/kg extract), test group I (50 mg/kg extract plus 23mg/kg indinavir sulphate) and test group II (Indinavir sulphate 23mg/kg). Physiological and biochemical tests assayed include body weight, fasting blood sugars, oral glucose tolerance test, fasting serum insulin levels, lipid profile, hepatic triglycerides, hepatic index, adipose tissue weights, serum uric acid and branched-chains amino acids, and fasting insulin levels.

The extract lowered the body weight of the rats by inhibiting the enzyme fatty acid synthase which is important in fatty acid synthesis ($P < 0.0001$). The extract also decreased fasting blood glucose and improved insulin sensitivity which was attributed to the enhanced glucose uptake by adipose tissue and muscle secondary to the

upregulation of GLUT-4 transporters expression ($P < 0.0001$). The liver indices were also reduced [hepatic triglycerides, liver weight, hepatic weight to body weight ratio ($P < 0.0001$)]. This was attributed to the improvement in insulin signalling. The extract lowered the serum lipids which was directly attributed to the improvement in insulin signalling, activation of PPAR α receptor which activates the gene for liver acyl-CoA oxidase increasing fatty acid oxidation in the liver, this action lowers hepatic lipids and in effect, serum lipid levels, controlling and preventing the hyperlipidaemia seen in diabetes. The extract caused a reduction of adipose tissue weights by activating AMPK pathway and preventing the accumulation of ectopic fat ($P < 0.0001$). A reduction of branched-chain amino acid was attributed to increased expression of the branched-chain ketoacid dehydrogenase complex, hence upregulation of adipose tissue branched-chain amino metabolizing enzyme. The reduction in serum uric acid exhibited by *Rotheca myricoides* (Hochst.) Steane & Mabb was due to reduced cellular oxidative stress.

The freeze-dried extracts of *Rotheca myricoides* (Hochst.) Steane & Mabb possessed significant antihyperglycemic and anti-dyslipidemic effects. In addition, it lowered body weight, adipose tissue weight, branched-chain amino acids, serum uric acid, as well as hepatic triglycerides and hepatic weight. These findings therefore demonstrate that *Rotheca myricoides* (Hochst.) Steane & Mabb possesses antidiabetogenesis effects in an animal model of type 2 diabetes.

CHAPTER ONE

1.1 Background

Type 2 diabetes (T2D) is a complex metabolic disorder essentially characterized by alterations in lipid metabolism, insulin resistance and pancreatic β-cell dysfunction (Podell *et al*., 2017).

The insulin resistance may affect whole-body physiology because insulin actions have different effects in various cells of the body (Mittelman *et al*., 1997). Insulin has both direct and indirect actions in cells with indirect effects predominating over direct effects (Titchenell *et al*., 2017). Direct actions include hepatic, skeletal muscle and white adipocyte insulin signalling (Perreault *et al*., 2018) while indirect actions include the inhibition of hepatic gluconeogenesis through reductions in hepatic acetyl CoA secondary to the suppression of lipolysis in white adipose tissue (WAT) leading to reductions in pyruvate carboxylase flux (Perry *et al*., 2015). The long-term persistence of type 2 diabetes fosters atherosclerosis (Gleissner *et al*., 2008) and leads to specific complications including cardiovascular disorders, retinopathy, neuropathy, cognitive and psychiatric disorders (Inzucchi *et al*.,2015).

There are currently no effective treatments available for type 2 diabetes as end organ damage still develops despite adequate glycaemic control, underscoring the need for newer more efficacious drugs for the management of T2D (Srinivasan and Ramarao, 2007). Approximately 80% of the population in some countries in Africa and Asia use herbal medicine (Wachtel-Galor and Benzie, 2011). Herbal remedies are, cheaper alternatives to conventional drug therapy, a useful source of new drugs e.g. metformin which was isolated from *Galega officinalis* (Rojas and Gomes, 2013), used together with the conventional therapy and effective in the treatment of various conditions (Bent, 2008). This study was aimed at investigating the antidiabetogenesis effects of *Rotheca myricoides* (Hochst.) Steane & Mabb on a rat model of Type 2 diabetes.

1.2 Problem statement

Type 2 diabetes accounts for 90% of diabetes with obesity being the most common risk factor for the development of T2D (Ghasemi and Jeddi, 2017). Obesity is associated with dyslipidaemia, hypertension and insulin resistance (Gheibi *et al.*, 2017). The incidence and prevalence of diabetes mellitus is rising at a rapid rate (Guthrie and Guthrie, 2004) with the International Diabetes Federation (IDF) projecting an increase in the number of patients affected from an estimated 285 million in 2010 to 438 million by 2030 (IDF Diabetes Atlas Group, 2015).

The WHO projection of fatalities from disease over the next ten years suggests that in Africa alone, 28 million people will succumb to a chronic disease. Deaths attributed to chronic illnesses are projected to increase by 27%, a rate over 4-fold higher than those attributed to infectious disease, childbirth and nutritional deficiency, which are projected to increase by 6%. Most significantly, the fatality of diabetes is projected to rise by 42% (Moszynski, 2006).

In low-income countries, individuals suffering from Diabetes have great difficulty in accessing

the appropriate medication to control their condition and many end up spending fortunes in dealing with the complications of the disease (Smith-Spangler et al., 2012). In many low-income

countries, there is an emerging trend of use of alternative and complementary medicine (ACM)

as patients attempt to improve the prognosis of disease with the least financial strain. Additionally, ACMs have attracted academic and economic interest because of the wide-spread and increasing popularity of use and the promise of novel therapeutic agents (Kasper and Giovannucci, 2006).

1.3 Study justification

Type 2 diabetes affects different cells in the body hence the need to study its effect on whole body physiology. Current treatment modalities do not cure or reverse the progression of the disease as end organ damage still develops despite adequate glycaemic control, underscoring the need for newer more efficacious drugs for the management of DM. Additionally, current modalities of treatment of Type 2 Diabetes have a number of adverse effects that include severe hypoglycaemia for Sulfonylureas, lactic acidosis for Metformin, weight gain and fluid retention for the Thiazolidinediones, and flatulence and diarrhoea for the α-Glucosidase inhibitors

In addition, because Type 2 DM is a progressive disease, no single agent provides adequate glycaemic control over the long term because none of the current treatment agents reverses the progression of the disease. Over time, even dual therapy may not be sufficiently effective, and additional antidiabetic agents may be required. Considerable uncertainty exists regarding optimal treatment and there therefore exists a need for the development of new agents that lack the adverse effects of current agents yet reverse the progression of the disease (McIntosh et al., 2012). The plant *Rotheca myricoides* (Hochst.) Steane & Mabb is used in traditional medicine in the management of diabetes thus the need to investigate its efficacy.

1.4 Hypothesis

Freeze-dried extracts of *Rotheca myricoides* (Hochst.) Steane & Mabb do not possess significant antidiabetogenesis effects.

1.5 Objectives

The general objective of this study was to evaluate the antidiabetogenesis effects of freeze-dried extracts of *Rotheca myricoides* (Hochst.) Steane & Mabb in a diet and low dose streptozocin type 2 diabetes animal model**.**

1.5.1 Specific objectives

The specific objectives of this work were

1. To determine the antidiabetogenesis effects of freeze-dried extracts of *Rotheca myricoides* (Hochst.) Steane & Mabb by measuring fasting blood glucose levels, oral glucose test and lipid profile.
2. To evaluate the effects of freeze-dried extracts of *Rotheca myricoides* (Hochst.) Steane & Mabb on metabolic markers by measuring the levels of hepatic triglyceride, serum uric acids levels and serum branched chain amino acids.
3. To investigate the physiological effects of freeze-dried extracts of *Rotheca myricoides* (Hochst.) Steane & Mabb on the following variables; body weight, adipose tissue weights and hepatic index (liver weight: Body weight Ratio)

1.6 Research question

1. What are the antidiabetogenesis effects of freeze-dried extracts of *Rotheca myricoides* (Hochst.) Steane & Mabb by measuring fasting blood glucose levels, oral glucose test and lipid profile?
2. What are the effects of freeze-dried extracts of *Rotheca myricoides* (Hochst.) Steane & Mabb on metabolic markers by measuring the levels of hepatic triglyceride, serum uric acids levels and serum branched chain amino acids?
3. What are the physiological effects of freeze-dried extracts of *Rotheca myricoides* (Hochst.) Steane & Mabb on the following variables; body weight, adipose tissue weights and hepatic index (liver weight: Body weight Ratio)?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Rotheca myricoides* (Hochst.) Steane & Mabb plant

Rotheca myricoides (Hochst.) Steane & Mabb (formerly known as *Clerodendrum myricoides)* (Steane and Mabberley, 1998), is a plant belonging to the genus Rotheca the largest genus of the family Verbenaceae (Bashwira and Hootele, 1988). The genus is native to tropical and warm temperate regions of the world. Most species occur in Africa and Southern Asia with a few species found in some parts of America and Australia. Plants belonging to this genus are widely used as herbal medicine in Africa and Asia in the management of diabetes mellitus. *Rotheca capitalum* (A. A. Adeneye *et al*., 2008b), *Rotheca phlomidis* (Mohan Maruga Raja, 2010) and *Rotheca glandulosum* (Jadeja *et al*., 2009) have all been shown to have hypoglycaemic and lipid lowering properties.

Rotheca myricoides (Hochst.) Steane & Mabb is used for management of diabetes in the lower eastern part of Kenya (Kitui, Machakos and Makueni Counties, Kenya) that is mainly inhabited by the Kamba community. A decoction is prepared by boiling the leaves and a cup (250 ml) taken daily (Keter and Mutiso, 2012). Other ethnobotanical uses of *Rotheca myricoides* (Hochst.) Steane & Mabb include epilepsy, arthritis, typhoid, cough, eye problems, tonsillitis, rheumatism, gonorrhoea (Moshi *et al*., 2012), cancer (Esubalew *et al*., 2017), malaria (Mukungu *et al*., 2016), dysmenorrhea, sterility, impotence, coughs, furunculosis, inflammation, and snakebites (Richard *et al*., 2011). No specific research has been done on the efficacy of *Rotheca myricoides* (Hochst.) Steane & Mabb in diabetes mellitus as well as the mechanism of action of its putative antidiabetogenesis effects (Winzell & Ahrén, 2004).

Figure 2.1: Image of *Rotheca myricoides* (Hochst.) Steane & Mabb (Harrison, 2009).

2.2 Phytochemistry screening of *Rotheca myricoides* (Hochst.) Steane & Mabb.

Phytochemical screening by use of dichloromethane (1:1) and methane (100%) on roots extracts of *Rotheca myricoides* (Hochst.) Steane & Mabb revealed the presence of phenolic compounds, steroids, alkaloids, saponins terpenes and banthraquinones (Jeruto *et al*., 2011). Chromatographic separation of dichloromethane/methane (1:1) whole plants extracts of *Rotheca myricoides* (Hochst.) Steane & Mabb yielded phenylpropanoid glycoside (Esatu *et al*., 2015)

2.3 Classification and diagnosis of diabetes mellitus

Diabetes mellitus is a syndrome of impaired carbohydrate, fat, and protein metabolism caused by either lack of insulin secretion or decreased sensitivity of the tissues to insulin. Insulin lack or resistance prevents efficient uptake and utilization of glucose by most cells in the body except the brain cells (Petro *et al*., 2004). The vast majority of cases of diabetes are classified into two broad etiopathogenetic categories (Menke et al., 2015). In one category, type 1 diabetes (due to autoimmune β-cell destruction), there is an absolute deficiency of insulin secretion. In the other, much more prevalent category, type 2 diabetes (due to a progressive loss of β-cell insulin secretion), there is a combination of resistance to insulin and an inadequate compensatory insulin secretory response (Chen *et al*., 2012). Other specific types of diabetes include, gestational diabetes mellitus (GDM) (diabetes diagnosed in the second or third trimester of pregnancy that was not clearly overt diabetes prior to gestation) (Mayfield, 1998), monogenic diabetes syndromes such as neonatal diabetes and maturity-onset diabetes of the young [MODY] (Dabelea *et al*., 2014), diseases of the exocrine pancreas such as cystic fibrosis (Skyler *et al*., 2017), and drug or chemical-induced diabetes e.g. with glucocorticoid use (Insel *et al*., 2015).

The diagnostic criteria for diabetes include a fasting plasma glucose level ≥126 mg/dL (≥7.0 mmol/L), 75 g OGTT 2-h value ≥200 mg/dL (≥11.1 mmol/l), Random plasma glucose level ≥200 mg/dL (≥11.1mmol/L), or HbA1c ≥ 6.5% (48mmol/mol). Re-examination is conducted at another date, and diabetes mellitus is diagnosed if 'diabetic type' is reconfirmed (Insel *et al*., 2015).

2.4 Pathophysiology of type 2 diabetes mellitus

Type 2 diabetes is characterized by dysregulation of carbohydrate, lipid and protein metabolism, and results from impaired insulin secretion, insulin resistance or a combination of both (DeFronzo, 2004). Insulin resistance, (often associated with obesity), and insulin secretion defects are the major risk factors for the development of type 2 diabetes (Cnop *et al.*, 2005). Increased caloric intake and decreased energy expenditure are important determinants of insulin resistance (Pan and Storlien, 1993). Nutrient composition, specifically increased amounts of dietary fat (particularly saturated fat), are important in the development of obesity, insulin resistance, β-cell dysfunction, and glucose intolerance (Hu *et al.*, 2001). A progressive decrease of β-cell function leads to glucose intolerance, which is followed by type 2 diabetes that worsens with time. In addition, aging is sometimes associated with reduction in the responsiveness of β cells to insulin production hence the glucose intolerance seen in old age (Gerich, 1999).

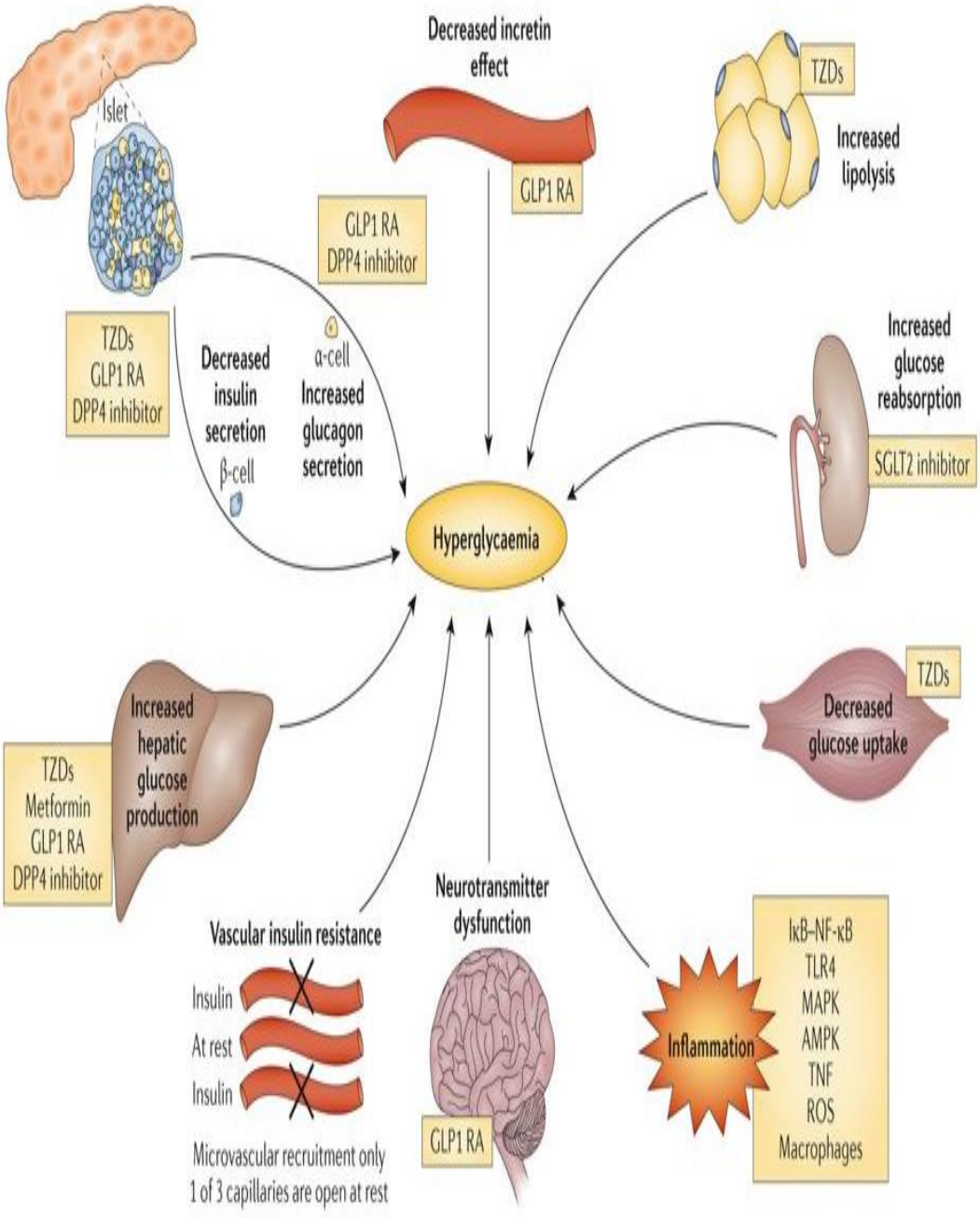


Figure 2.2: Diagram showing the Pathophysiology of Type 2 diabetes mellitus (Chen *et al*., 2012).

2.5 Mechanism of action of hypoglycaemic herbs

Majority of the traditional remedies act via enhancing glucose uptake by adipose and muscle tissues (Shepherd and Kahn, 1999), other mechanisms include stimulating insulin secretion, inhibiting glucose absorption from intestine or by inhibiting hepatic glucose production (Hui *et al*., 2009). The various mechanisms are summarized in Figure 3 below.

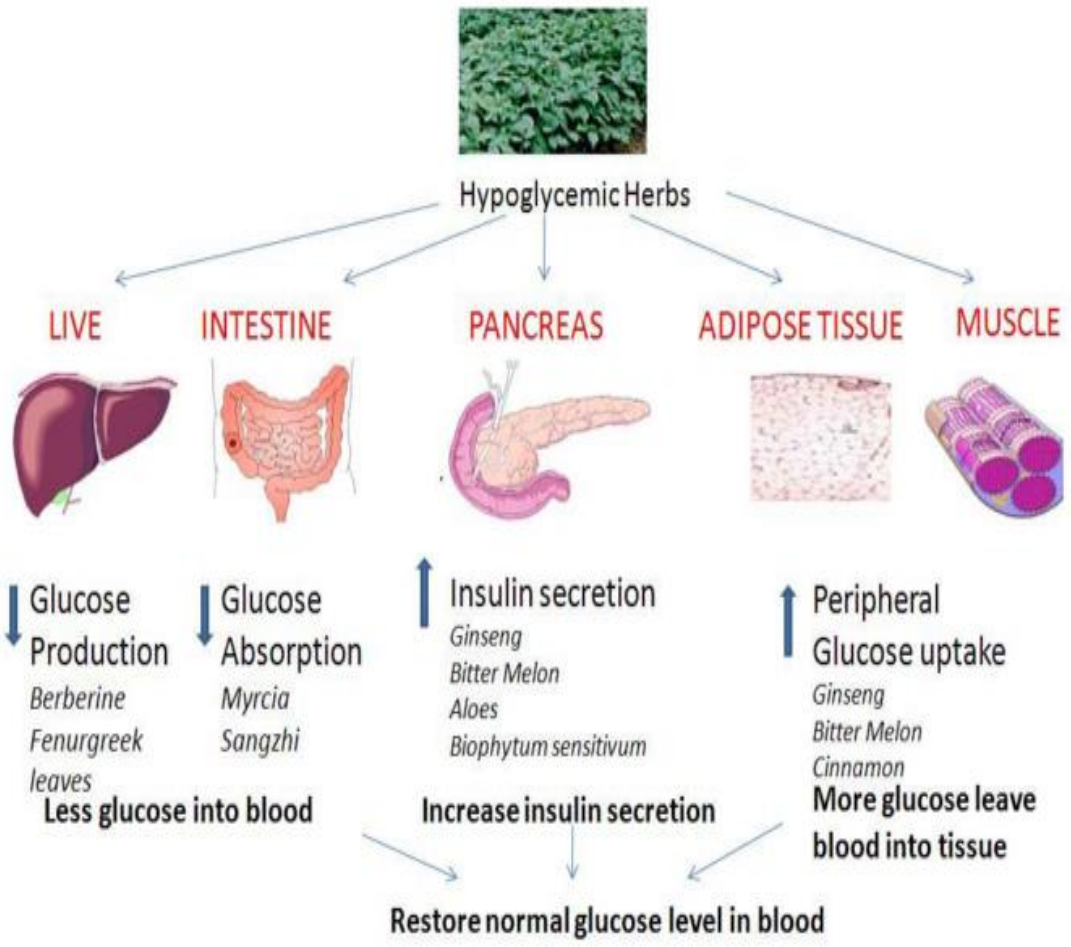


Figure 2.3: Diagram showing the mechanisms of hypoglycaemic herbs (Hui *et al*., 2009).

2.6 Induction models of type 2 diabetes mellitus

Animal models of Type 2 diabetes mellitus are either spontaneous or induced by surgical manipulation, diet, chemical or by combination of any of these methods (Srinivasan and Ramarao, 2007). Transgenic or knock out animals are also used where it is possible to study the effects of gene mutations in development of diabetes. The model is however costly to produce and maintain (King, 2012). Animal models of Type 2 DM often include genetic mutations or manipulation induction by a high sugar and high fat diet (Dong *et al*., 2014). A high sugar and high fat diet is the diet of choice for the induction of type 2 diabetes (Gan *et al*., 2014). Such a diet results in the development of obesity, hyperinsulinemia, and insulin resistance (Flanagan *et al*., 2008). Initial β-cell dysfunction that mimics that in type 2 diabetes mellitus is achieved by a low dose of either alloxan or streptozocin administered intraperitoneally (IP). However, streptozocin (STZ) has been observed to be a better chemical inducer than alloxan (Szkudelski, 2001). Rat and mice models are most commonly used for type 2 diabetes (King, 2012).

2.7 Measurement of branch chain amino acids using Raman spectroscopy

Raman spectroscopy is one of the vibrational spectroscopic techniques used to provide information on molecular vibrations and crystal structures. It relies on inelastic scattering of monochromatic light, usually from a laser in the visible, near infrared or near ultraviolent rays. The laser light interacts with molecular vibrations in the system, resulting in the energy of the laser photons being shifted up and down. The shift in energy gives information about the vibrational modes in the system (Joya and Sala, 2015). Since vibrational frequencies are specific to a molecule's chemical bonds and symmetry, Raman provides a fingerprint to identify molecules (Ferrari, 2007). Raman spectroscopy involves shining a laser light onto the sample of interest and the wavelength-shifted (Raman scattered) radiation collected, dispersed in a spectrometer and recorded. This radiation emanates from radiative vibrational relaxations in excited molecules in the sample and thus the resultant spectral profile is unique to them. This spectroscopic technique is increasingly generating a lot of interest in diabetes detection

with spectral bands associated with some biomolecules acting as biomarkers (Joya and Sala, 2015).

Some of the biomolecules that have been demonstrated using Raman spectroscopy to have a great potential in the detection and monitoring of type 2 diabetes include blood glucose (Guevara *et al*., 2018) haemoglobin (Gong *et al*., 2018) lipids in erythrocyte membranes, and branched chain amino acids (Birech *et al*., 2017).

Lipid species have for a long time been claimed to be the underlying cause of insulin resistance, but accumulating evidence shows that branched-chain amino acids (BCAAs) (valine, isoleucine, and leucine) and by-products of their catabolism (for example Glutamate, Alanine, and C5 and C3 acyl-carnitines) have a stronger correlation to insulin resistance than most common lipid species (Newgard, 2012). The rise in BCAA levels is an indicator of the onset of insulin resistance, and can be used to predict the pre-diabetic to diabetic hence initiate early intervention to reverse the progression of type 2 diabetes mellitus (Huffman *et al*., 2009). Its use (i.e., Raman spectroscopy) in comparative efficacy studies of antidiabetic medications, both conventional and traditional, has been reported elsewhere (Birech *et al*., 2017).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Extraction of *Rotheca myricoides* (Hochst.) Steane & Mabb and diet preparation

Rotheca myricoides (Hochst.) Steane & Mabb was collected from its natural habitat in Machakos county, Kenya its identity verified at the University of Nairobi herbarium and a voucher specimen deposited therein (BMC 2017/01). The whole plant was used in the study. The plant was air dried for a week and ground into a fine powder using a standard laboratory mill. The powder was then macerated in distilled water for twenty-five (25) minutes in a weight/volume ratio of 1:10. The resulting suspension was then filtered in succession using cotton wool and Whatman® filter paper. The resulting filtrate was then lyophilized to obtain the freeze-dried extract which was then weighed and placed in amber coloured sample containers and placed in a standard in a laboratory refrigerator.

3.2 Animal procedures and protocols

Sixty-forty (64), freshly-weaned (four-week old) Sprague Dawley rats (thirty-two males and thirty-two females), weighing 60-110g were obtained from the Department of Zoology, University of Nairobi. They were grouped-housed in the animal house within the department of medical physiology under the following ambient conditions: room temperature of 23 ± 2°C, relative humidity 30-50% and a 12-hr light-dark cycle. The animals were habituated to both the experimenter and the environmental conditions for seven (7) days prior to the start of the study.

Diabetes was induced in all the experimental animals using a combined dietary and chemical approach. The experimental animals were fed on a high fat and high fructose (20% weight/volume fructose solution) diet *ad libitum* for six (6) weeks after which a low dose of streptozocin (30mg/kg) was administered intraperitoneally to all rats at day 42 of the experimental period. Administration of the high fat and high fructose

diet continued for a further two weeks after administration of streptozocin as outlined in the protocol by Gheibi *et al*. (2017).

Forty-five (45) grams of solid cooking fat were added (Frymate® vegetable cooking oil, Pwani oil products) to 225g of standard chow pellets (energy content due carbohydrate:70%, Protein: 20%, Fat: 10%) (Unga Feeds Limited, Nairobi) and monosodium glutamate (MSG) (0.8%) (Oshwal Flavours Limited, Nairobi, Kenya) was added to improve the palatability. The mixture was gently heated over low heat for 20 minutes with constant mixing. The chow was drained of excess fat, cooled and weighed to ensure achievement of 15% fat content. It was then stored in air tight containers for later use. Twenty (20) grams of sugar (99% fructose) (Martinez Nieto, S.A., Spain) was dissolved in 100ml water to form a 20% fructose solution. This was prepared daily and fed to the rats *ad libitum* throughout the experiment.

3.3 Determination of the efficacy of the *Rotheca myricoides* extract

Forty (40) rats were randomly assigned to the negative control (normal saline), low dose test (50mg/kg extract), high dose test (100mg/kg extract) and positive control (Pioglitazone 20mg/kg) groups (n= 10 per group). The respective treatments were administered daily by oral gavage. The high dose and low dose extract solutions were freshly prepared.

3.4 Measurement of fasting blood glucose and body weight of experimental rats

The rats were weighed weekly and BMI was estimated by dividing body weight (kg) the square of the naso-anal length (m^2) at the start of the study. Fasting blood glucose (FBG) was assayed on a weekly basis using a glucometer (StatStrip Xpress® Nova Biomedical, Waltham MA, USA) with the respective blood samples being obtained using lateral tail vein blood sampling (Topical Lidocaine having been applied ten (10) minutes before the procedure to ease pain/stress associated with the test) (Lee and Goosens, 2015).

3.5 Determination of insulin sensitivity using oral glucose tolerance test (OGTT)

Oral glucose tolerance tests were performed on days 28 and 56 of the experimental period using the protocol described by Barret (Barrett, 2002). Briefly, the rats were fasted for 6-8 hours prior to the test. The baseline blood glucose was then determined using the procedure described previously after which a loading dose of glucose (2 g/kg) was administered to each rat by oral gavage. Blood glucose levels were then determined at 30, 60, 90 and 120 minutes after administration of the loading dose of glucose.

3.6 Measurement of fasting serum insulin levels

The serum insulin level was determined using the enzyme-linked immunosorbent assay (ELISA) method using a rat insulin kit (Hangzhou Sunlong Biotech Co. Ltd., China).

The homeostasis model assessment of Insulin Resistance (HOMA-IR) was calculated using the following equation:

HOMA-IR = Insulin (mU/L) *glucose (mg/dl)/405.

3.7 Quantification of lipid profile and serum uric acid levels

All rats were euthanized after an overnight fast by the administration of 1mg/kg of 20% Phenobarbital intraperitoneally at the end of week 8, i.e. day 56. Blood was then collected by cardiac puncture, allowed to clot then centrifuged at 1500 revolutions per minute for ten (10) minutes and the serum obtained transferred into vacutainers. The sera were then transported to the Department of Clinical Chemistry, University of Nairobi, where serum uric acid, serum triglyceride, total cholesterol, low density lipoprotein cholesterol and high density lipoprotein cholesterol were performed.

3.8 Estimation of hepatic triglycerides levels in rats

After euthanization as described above, a midline body was on each rat was made to expose the abdominal cavity and the liver excised. The liver samples were then deep-frozen at - 90^{O} C and, the hepatic triglycerides determined using the procedure by

Butler and Mailing (Butler et al., 1961). Briefly, two (2) g portions of the respective livers were homogenized in eight (8) millilitres of phosphate Buffer. One (1) ml of the resulting homogenate was then added to four (4) g of activated charcoal which had been pre-moistened with two (2) millilitres of chloroform. The resulting paste was then topped up with eighteen (18) millilitres of chloroform and gently shaken for 10 minutes after which it was filtered. The resulting filtrate was then was divided into 3 test tubes. One (1) ml of standard oil solution (1%) was pipetted into 3 additional test tubes. All the test tubes were placed in a water bath at 80°C and excess chloroform evaporated. Zero-point five (0.5) ml alcoholic potassium hydroxide was added to the first & second tube and 0.5 ml of 95% alcohol was added to the third tube containing the filtrate and the test tube containing the standard corn oil solution. The test tubes were maintained in water at 60°C for twenty (20) minutes after which 0.5 ml of 0.2 N sulphuric acid were added to each tube and the resulting mixtures heated in a water bath (100°C) for twenty (20) minutes. They were then cooled after which 0.1 ml sodium metaperiodate followed by 0.1 ml sodium arsenite were added. Five (5) ml of chromotropic acid were then added to each test tube after ten (10) minutes. The tubes were placed in a water bath (100°C) for half an hour. The optical densities at 540 nm were then determined using spectrophotometer. The optical densities obtained were then used to calculate the hepatic triglyceride content using the formula described by Butler *et al.* (1961) as follows:

$$Let\ R$$

$$= \left(\frac{Optical\ density\ (O.D)\ saponified\ unknown - O.D\ unsaponified\ unknown}{O.D\ saponified\ corn\ oil\ standard - O.D\ unsaponified\ corn\ oil\ standard}\right)$$

$$And\ A = volume\ of\ aliquot\ of\ chloroform\ extract\ in\ ml$$

$$(1\ ml\ was\ used\ in\ the\ present\ study).$$

Then triglyceride contents in milligram per gram of tissue

$$\frac{200}{A} \times R \times 0.05 = 10\frac{R}{A}$$

3.9 Determination of branched chain amino acids using Raman spectroscopy

A spectroscopic signature of each, branched chain amino acids (isoleucine, leucine, valine) and creatine monohydrate was acquired using pure crystals, to obtain a reference curve of Raman Spectroscopy. On day 56, blood was obtained for above assay. The animals were fasted for 6 hours before collection then anaesthetized. Anaesthesia was then confirmed by checking loss of the blink reflex. Blood was collected by lateral tail vein sampling and stored in sodium citrate vacutainers to prevent clotting. An assay was then done using a Raman spectroscopy.

Before commencement, the charge-coupled device (CCD) was cooled to -76 to prevent thermal noise from affecting the spectral readings. Grating was set at 600 BLZ, the range of spectral shift being centred at 1055.851 cm^{-1}. The detector was then set to make 10 readings and an average obtained, which was then displayed as the spectral chart to optimize the recordings. Individual reading was obtained by exposing the detector to the scattered light for seconds to optimize the amount of Raman scatter captured. The spectroscope was set to filter out cosmic rays by obtaining a background reading of the light conditions before spectral collection and then using this as a baseline for the spectral readings. Calibration of the machine was done with silicon and confirmed by obtaining Silicon's single peak at 520.5 nm. The laser beam was set at 785 nm during the procedure.

The sample was placed on the silver smear on the glass slide and brought to focus using a bright field microscopy. The objective lens of the microscope was fixed at a magnification of X10 with a numerical aperture of 0.50. A dark-field microscope was set before shooting the laser beam onto the sample. A dark room was used for recording all the spectral reading.

3.10 Mechanism of action of the freeze-dried extract of *Rotheca myricoides*

Twenty-four rats (24) were randomly assigned to the negative control (normal saline), positive control (50 mg/kg extract), test group I (50 mg/kg extract plus 23 mg/kg indinavir sulphate) and test group II (Indinavir sulphate 23 mg/kg). The inclusion of Indinavir sulphate was because it is a known antagonist of GLUT-4 (Hruz *et al*., 2002).

3.11 Ethical approval

This study was carried out in strict accordance with the recommendations in the Guide for care and Use of Laboratory Animals of the National Institutes of Health (Clark *et al*., 1997). The protocol was approved by the Biosafety, Animal use and Ethics committee Department of Veterinary Anatomy and Physiology, University of Nairobi (permit number 161). In addition, the experimental design obeyed the 4Rs (reduction, replacement, refinement and rehabilitation) of ethical animal experimental design (Guillen, 2012).

3.12 Data analysis

All the data obtained were expressed as mean ± SEM. The data was analysed using one-way ANOVA followed by Tukey's test in cases of significance. Analysis was performed using GraphPad Prism 7.0 (Graph Pad, USA). Principal component analysis was applied on a combined spectral data (Raman spectroscopy) set from the four experimental groups Principal component analysis utilizes spectral patterns in segregating between data sets.

CHAPTER FOUR

4.0 RESULTS

4.1 Baseline characteristics of the rats and percentage yield of the extract

The amount of freeze-dried extract of *Rotheca myricoides* (Hochst.) Steane & Mabb obtained per 100 g of dried whole plant was 12.45 g (Percentage Yield = 12.45 %). The mean body weight of all the rats at the beginning of the experiment was 102 ± 6.04 g. There were no statistically significant differences in the body weight between the four groups after randomization [100.4 ± 6.56 g (negative control) vs. 100.5 ± 3.92 g (low dose test) vs. 104 ± 5.18g (high dose test) vs. 106.4 ± 8.52g (positive control): $F(3,36) = 2.522$: $p = 0.00731$]. Table 4.1 shows the average baseline body weight and BMI of the rats in each group.

Table 4.1: Body weight and Body Mass index at Week 0 expressed as Mean ± SEM.

Variable	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Body weight (g)	100.4 ± 6.56	100.5 ± 3.92	104 ± 5.18	106.4 ± 8.52
Body Mass Index (g/cm^2)	0.39 ± 0.012	0.41 ± 0.020	0.40 ± 0.015	0.42 ± 0.018

4.2 Effects of the freeze-dried extract of *Rotheca myricoides* on body weight of the rats

There were no significant differences in the body weight between the four experimental groups at the beginning of the study [100.4 ± 6.56 grams (negative control) vs. 100.5 ± 3.92 grams (low dose test) vs. 104.1 ± 5.18 grams (high dose test) vs. 106.4 ± 8.52 grams (positive control): F (3, 36) = 1.508: p = 0.8853].There were no significant differences in the body weight between the four experimental groups at the end of Day 7: [120.9 ± 8.07 grams (negative control) vs. 121 ± 4.34 grams (low dose test) vs. 127.4 ± 8.23 grams (high dose test) vs. 125.5 ± 7.51 grams (positive control): F (3, 36) = 1.264: p = 0.9021].There were no significant differences in the body weight between the four experimental groups on Day 14: [150.4 ± 6.71 grams (negative control) vs. 141.3 ± 7.07 grams (low dose test) vs. 149.6 ± 12.06 grams (high dose test) vs. 155.8 ± 7.93 grams (positive control) : F (3,36) = 2.697: p = 0.7051].

There were no significant differences in the body weight between the four experimental groups on Day 21: [168.7 ± 6.97 grams (negative control) vs.147.6 ± 7.13 grams (low dose test) vs. 162.2 ± 13.44 grams (high dose test) vs. 160.5 ± 8.9 grams (positive control): F (3, 36) = 2.522: p = 0.4631].

There were no significant differences in the body weight between the four experimental groups on Day 28: [182.5 ± 8.74 grams (negative control) vs. 163 ± 7.44 grams (low dose test) vs. 171.4 ± 14.19 grams (high dose test) vs. 185.8 ± 8.81 grams (positive control). F (3, 36) = 2.98: p = 0.3820].

There were significant differences in the body weight between the four experimental groups on Day 35: [236.6 ± 5.28 grams (negative control) vs. 190.6 ± 10.52 grams (low dose test) vs. 211.3 ± 9.01 grams (high dose test) vs. 241 ± 6.78 grams (positive control). (3, 36) = 1.49: p = 0.0002]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the negative control and low dose test (50mg) (p = 0.0317) and the low dose test (50mg) and positive control (p = 0.0810).

There were significant differences in the body weight between the four experimental groups on Day 42: [277.6 ± 6.36 grams (negative control) vs. 231.4 ± 8.15 grams (low

dose test) vs. 258 ± 4.71 grams (high dose test) vs. 283.2 ± 8.93 grams (positive control). F (3, 36) = 1.162: $p < 0.0001$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the negative control and low dose test (50mg) ($p = 0.0004$) and the low dose test (50mg) and positive control ($p < 0.0001$).

There were significant differences in the body weight between the four experimental groups on Day 49: [321.2 ± 5.14 grams (negative control) vs. 275.8 ± 9.68 grams (low dose test) vs. 292 ± 4.55 grams (high dose test) vs. 326.8 ± 6.61 grams (positive control). F (3, 36) = 1.837: $p < 0.0001$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the negative control and low dose test (50mg) ($p = 0.0002$), the negative control and high dose test (100mg) ($p = 0.0215$), low dose test (50mg) and positive control ($p < 0.0001$) and high dose test (100mg) and positive control ($p = 0.0047$). There were significant differences in the body weight between the four experimental groups on Day 56: [343.6 ± 4.31 grams (negative control) vs. 299.5 ± 14.82 grams (low dose test) vs. 306.3 ± 4.16 grams (high dose test) vs. 340 ± 6.3 grams (positive control). F (3, 36) = 4.257: $p = 0.0008$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the negative control and low dose test (50mg) ($p = 0.0047$), negative control and high dose test (100mg) ($p = 0.0221$), low dose test (50mg) and positive control ($p < 0.0001$) and, high dose test (100mg) and positive control ($p = 0.0026$). The graphical presentation of the experimental data is shown in Figure 4.1 and results of Tukey's HSD post-hoc test, after a One-way ANOVA on body weight are shown in Table 4.2.

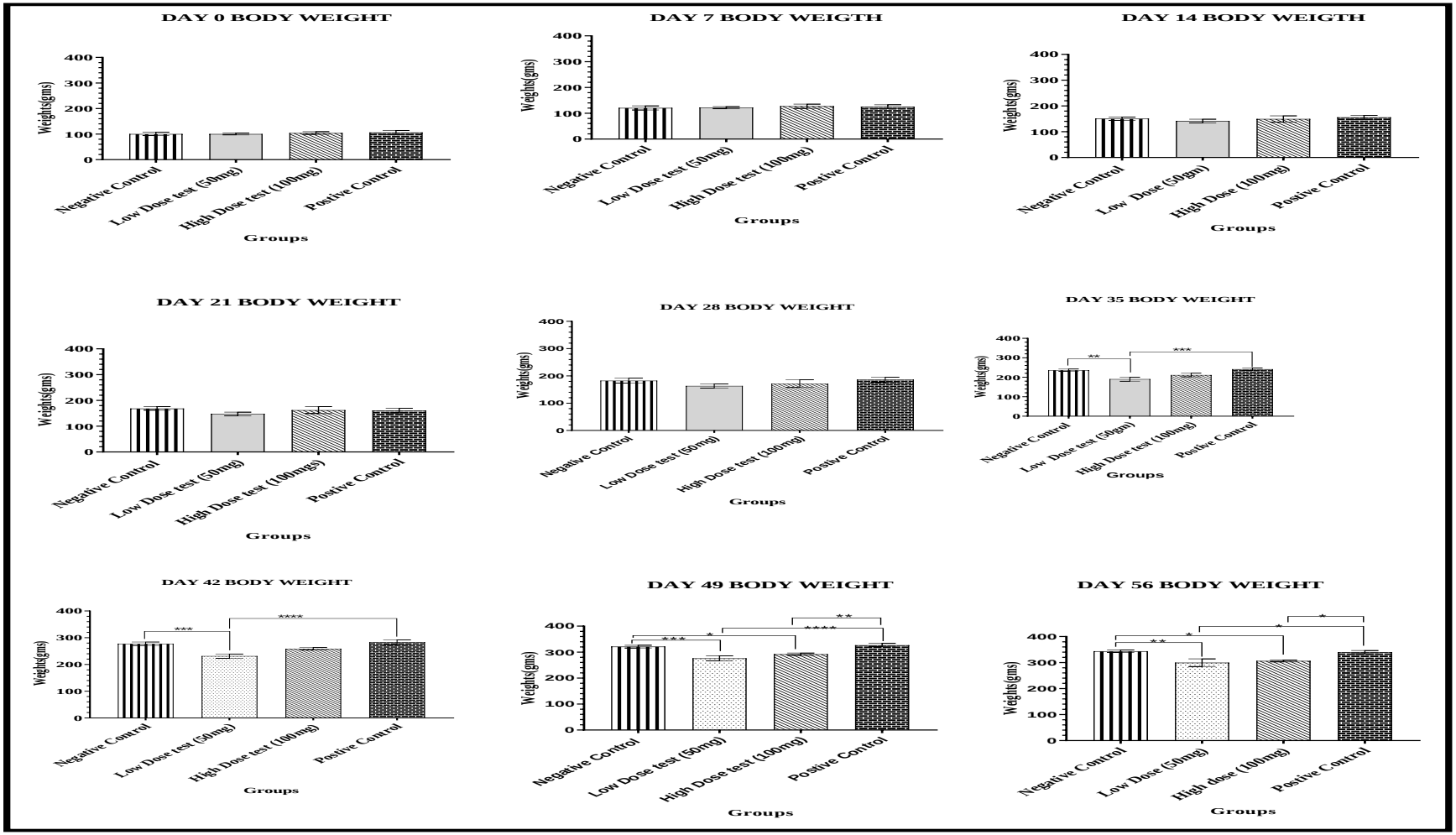


Figure 4.1: Graphs showing weekly mean body weight (g) over the 8-week experimental period expressed as Mean ± SEM.

Table 4.2: Results of Tukey's HSD post hoc test p values, after a One-way ANOVA on body weight.

Tukey's HSD Test	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	Day 49	Day 56
Negative control vs Low dose test (50mg)	>0.9999 (ns)	>0.9999 (ns)	0.8807 (ns)	0.4024 (ns)	0.5392 (ns)	0.0317 (*)	0.0004 (***)	0.0002 (***)	0.0047 (***)
Negative control vs High dose test (100mg)	0.9749 (ns)	0.9198 (ns)	>0.9999 (ns)	0.9606 (ns)	0.8660 (ns)	0.2015 (ns)	0.2696 (ns)	0.0215 (*)	0.0221 (*)
Negative control vs Positive control	0.9068 (ns)	0.9693 (ns)	0.9721 (ns)	0.9269 (ns)	0.9955 (ns)	0.9467 (ns)	0.9465 (ns)	0.9371 (ns)	0.9912 (ns)
Low dose test (50mg) vs High dose test (100mg)	0.9771 (ns)	0.9361 (ns)	0.9059 (ns)	0.6982 (ns)	0.9389 (ns)	0.0576 (ns)	0.0526 (ns)	0.3463 (ns)	0.9453 (ns)
Low dose test (50mg) vs. Positive control	0.9117 (ns)	0.9783 (ns)	0.6472 (ns)	0.7693 (ns)	0.4026 (ns)	0.0180 (*)	<0.0001 (****)	<0.0001 (****)	<0.0001 (****)
High dose test (100mg) vs. Positive control	0.9942 (ns)	0.9977 (ns)	0.9589 (ns)	0.9993 (ns)	0.7476 (ns)	0.0810 (ns)	0.0966 (ns)	0.0047 (**)	0.0026 (**)

Ns-Not significant, *- p < 0.05, **- p < 0.01, *- p < 0.001, ****- p < 0.0001.**

4.3 Outcome of freeze-dried extract of *Rotheca myricoides* on fasting blood glucose

There were no significant differences in the fasting blood glucose between the four experimental groups at the beginning of the experiment [4.42 ± 0.10 mmol/L (negative control) vs. 4.41 ± 0.08 mmol/L (low dose test) vs. 4.43 ± 0.13 mmol/L (high dose test) vs. 4.37 ± 0.10 mmol/L (positive control): F (3,36) = 0.5918: p = 0.6244]. There were no significant differences in the fasting blood glucose between the four experimental groups on Day 7: [4.25 ± 0.04 mmol/L (negative control) vs. 4.08 ± 0.06 mmol/L (low dose test) vs. 4.15 ± 0.06 mmol/L (high dose test) vs. 4.10 ± 0.04 mmol/L (positive control): F (3, 36) = 0.09901: p = 0.9601].

On Day 14, there were significant differences in fasting blood glucose between the four experimental groups [4.64 ± 0.80 mmol/L (negative control) vs. 4.14 ± 1.10 mmol/L (low dose test) vs. 4.36 ± 0.11 mmol/L (high dose test) vs. 4.02 ± 0.15 mmol/L (positive control): F (3,36) = 0.6251: p = 0.0029]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the negative control and low dose test (50mg) (p = 0.0198), the negative control and positive control (p = 0.0028).

There were significant differences in fasting blood glucose between the four experimental groups On Day 21: [5.06 ± 0.08 mmol/L (negative control) vs. 4.17 ± 0.10 mmol/L (low dose test) vs. 4.50 ± 0.12 mmol/L (high dose test) vs. 4.12 ± 0.12 mmol/L (positive control): F (3,36) = 0.3253: p < 0.0001]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the negative control and low dose test (50mg) (p < 0.0001), the negative control and high dose test (100mg) (p = 0.0034) and the negative control and positive control (p < 0.0001)

On Day 28, there were significant differences in fasting blood glucose between the four experimental groups [6.14 ± 0.18 mmol/L (negative control) vs. 4.65 ± 0.14 mmol/L (low dose test) vs. 5.17 ± 0.15 mmol/L (high dose test) vs. 4.66 ± 0.11 mmol/L (positive control). F (3, 37) = 0.3531: p < 0.0001]. Post -hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the

negative control and low dose test (50mg) ($p = 0.0026$), the negative control and high dose test (100mg) ($p = 0.0142$) and the negative control and positive control ($p = 0.0012$).

On Day 35, there were significant differences in fasting blood glucose between the four experimental groups [6.50 ± 0.11 mmol/L (negative control) vs. 4.62 ± 0.13 mmol/L (low dose test) vs. 5.25 ± 0.16 mmol/L (high dose test) vs. 4.33 ± 0.89 mmol/L (positive control). $F (3, 34) = 0.2618$: $p < 0.0001$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the negative control and low dose test (50mg) ($p < 0.0001$), the negative control and high dose test (100mg) ($p = 0.0008$), the negative control and positive control ($p < 0.0001$) and the high dose test (100mg) and positive control ($p = 0.0012$).

On Day 42, there were significant differences in fasting blood glucose between the four experimental groups [7.19 ± 0.11 mmol/L (negative control) vs. 4.02 ± 0.10 mmol/L (low dose test) vs. 4.43 ± 0.16 mmol/L (high dose test) vs. 4.11 ± 0.13 mmol/L (positive control). $F (3, 36) = 0.8722$: $p < 0.0001$]. Post -hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the negative control and low dose test (50mg) ($p < 0.0001$), the negative control and high dose test (100mg) ($p < 0.0001$) and the negative control and positive control ($p < 0.0001$).

On Day 49, there were significant differences in fasting blood glucose between the four experimental groups [7.80 ± 0.24 mmol/L (negative control) vs. 3.78 ± 0.09 mmol/L (low dose test) vs. 3.92 ± 0.12 mmol/L (high dose test) vs. 3.86 ± 0.15 mmol/L (positive control). $F (3, 36) = 0.8722$: $p < 0.0001$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the negative control and low dose test (50mg) ($p < 0.0001$), the negative control and high dose test (100mg) ($p < 0.0001$), the negative control and positive control ($p < 0.0001$).

On Day 56, there were significant differences in fasting blood glucose between the four experimental groups [8.06 ± 0.15 mmol/L (negative control) vs. 3.54 ± 0.09 mmol/L (low dose test) vs. 3.73 ± 0.11 mmol/L (high dose test) vs. 3.68 ± 0.09 mmol/L (positive control). $F (3, 37) = 0.8802$: $p < 0.0001$]. Post -hoc statistical analysis using

Tukey's multiple comparisons test revealed significant differences between the negative control and low dose (50mg) ($p < 0.0001$), the negative control and high dose test (100mg) ($p < 0.0001$), the negative control and positive control ($p < 0.0001$). The graphical representations of these results are shown in Figure 4.2, Table 4.3 and Table 4.4.

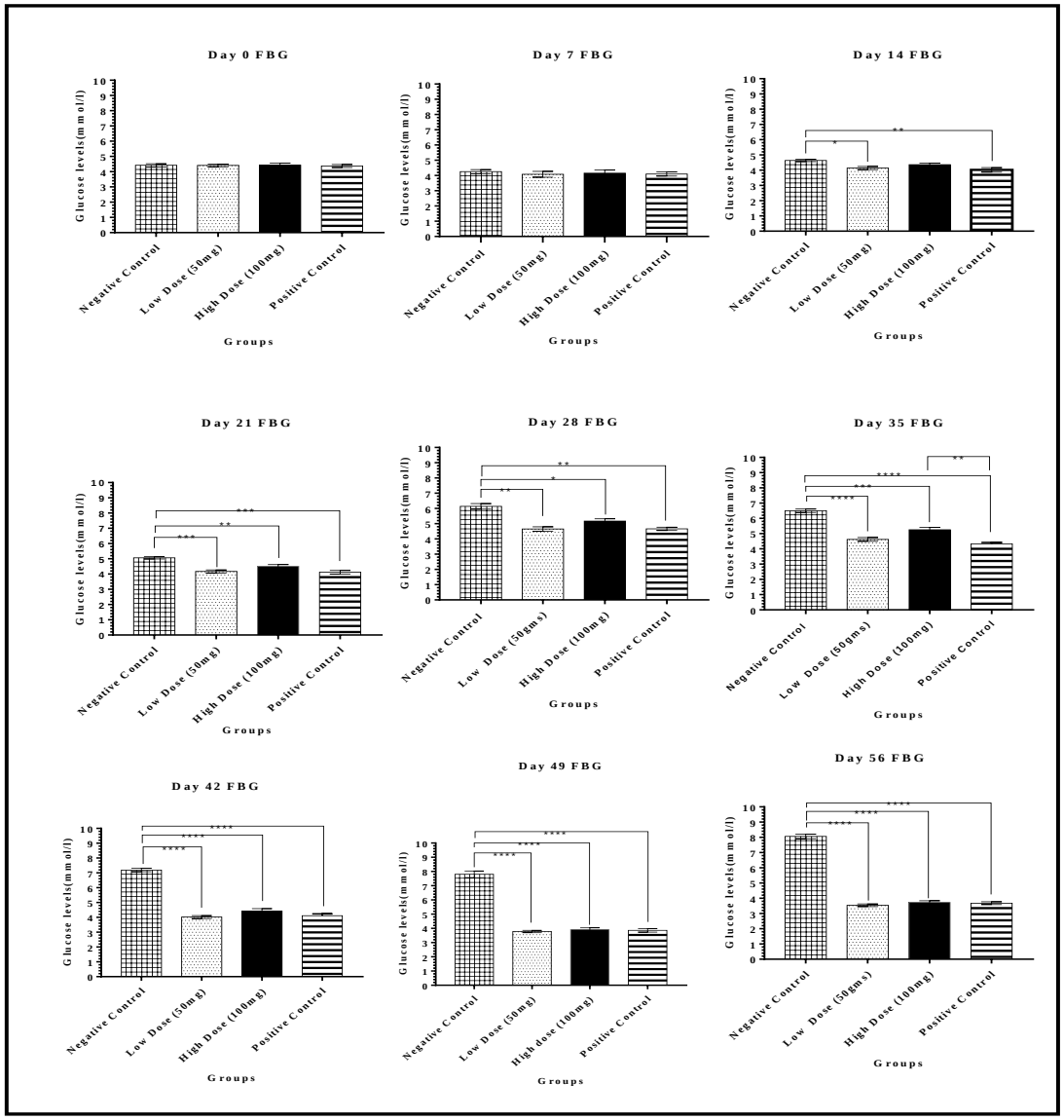


Figure 4.2: Graphs depicting fasting blood glucose levels (mmol/L) at weekly intervals during the experimental period.

Expressed as mean ± SEM. (*- $p < 0.05$, **- $p < 0.01$, ***- $p < 0.001$, ****- $p < 0.0001$).

Table 4.3: Fasting blood glucose levels recorded weekly during the study expressed as mean ± SEM.

Days	Negative control	Low dose (50mg)	High dose (100mg)	Positive control
Day 0	4.42 ± 0.10	4.41 ± 0.08	4.43 ± 0.13	4.37 ± 0.10
Day 7	4.25 ± 0.04	4.08 ± 0.06	4.15 ± 0.06	4.10 ± 0.04
Day 14	4.64 ± 0.08	4.14 ± 0.10	4.36 ± 0.11	4.02 ± 0.15
Day 21	5.06 ± 0.08	4.17 ± 0.10	4.50 ± 0.12	4.12 ± 0.12
Day 28	6.14 ± 0.18	4.65 ± 0.14	5.17 ± 0.15	4.66 ± 0.11
Day 35	6.50 ± 0.11	4.62 ± 0.13	5.25 ± 0.16	4.33 ± 0.89
Day 42	7.19 ± 0.11	4.02 ± 0.10	4.43 ± 0.16	4.11 ± 0.13
Day 49	7.80 ± 0.24	3.78 ± 0.09	3.92 ± 0.12	3.86 ± 0.15
Day 56	8.06 ± 0.15	3.54 ± 0.09	3.73 ± 0.11	3.68 ± 0.09

Table 4.4: Results of Tukey's HSD post hoc test, after a One-way ANOVA on fasting blood glucose levels

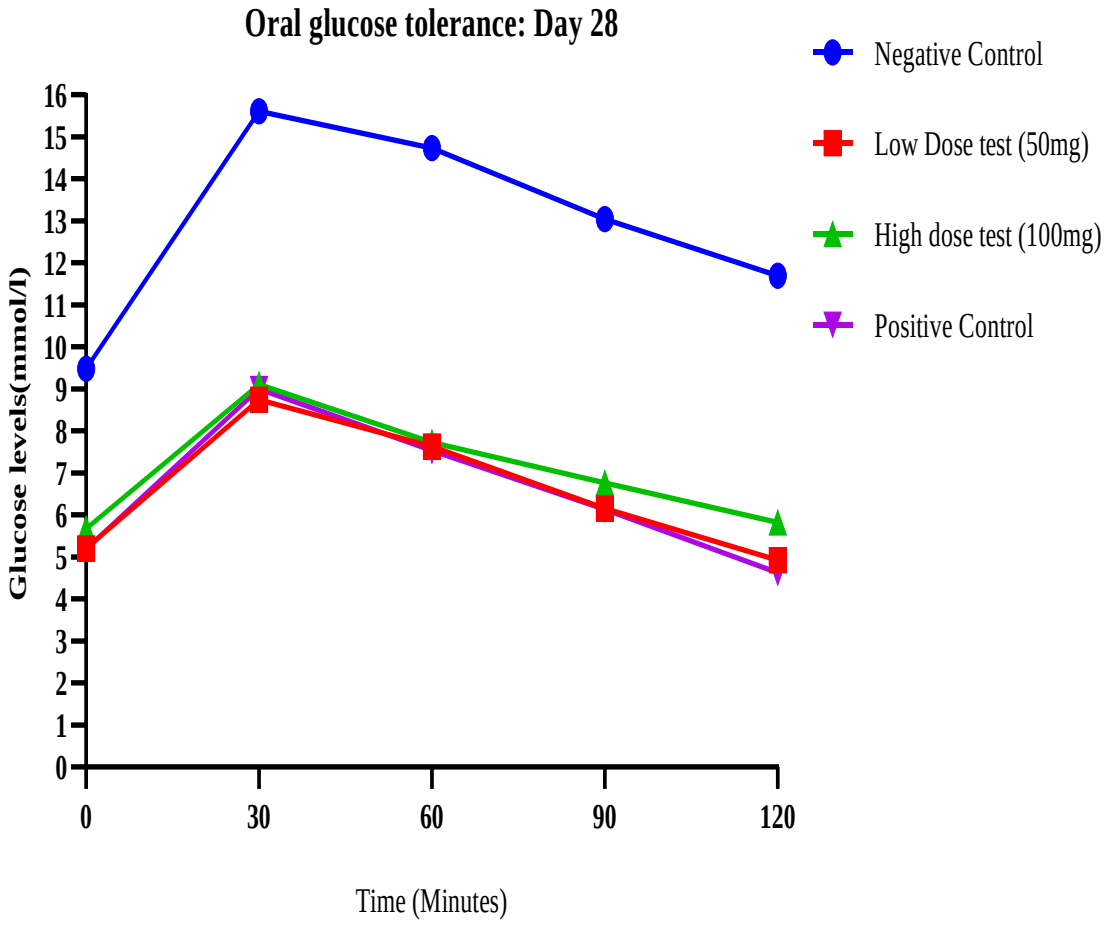
Tukey's HSD Test	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	Day 49	Day 56
Negative control vs. Low dose (50mg)	0.9999 (ns)	0.1288 (ns)	0.0198 (*)	< 0.0001 (****)	0.0026 (**)	< 0.0001 (****)	< 0.0001 (****)	< 0.0001 (****)	< 0.0001 (****)
Negative control vs. High dose (100mg)	0.9999 (ns)	0.5538 (ns)	0.3265 (ns)	0.0034 (**)	0.0142 (*)	0.0008 (***)	< 0.0001 (****)	< 0.0001 (****)	< 0.0001 (****)
Negative control vs. Positive control	0.9875 (ns)	0.2118 (ns)	0.0028 (**)	<0.0001 (****)	0.0012 (**)	< 0.0001 (****)	< 0.0001 (****)	< 0.0001 (****)	< 0.0001 (****)
Low dose(50mg) vs. High dose (100mg)	0.9992 (ns)	0.7905 (ns)	0.5353 (ns)	0.1404 (ns)	0.1701 (ns)	0.1252 (ns)	0.1205 (ns)	0.9157 (ns)	0.6252 (ns)
Low dose(50mg) vs. Positive control	0.9935 (ns)	0.9934 (ns)	0.8807 (ns)	0.9869 (ns)	0.9999 (ns)	0.4765 (ns)	0.9582 (ns)	0.9824 (ns)	0.8096 (ns)
High dose (100mg) vs. Positive control	0.9789 (ns)	0.9105 (ns)	0.1746 (ns)	0.0701 (ns)	0.1145 (ns)	0.0012 (**)	0.2975 (ns)	0.9924 (ns)	0.9887 (ns)

Ns-Not significant, *- $p < 0.05$, **- $p < 0.01$, ***- $p < 0.001$, ****- $p < 0.0001$

4.4 Results of *Rotheca myricoides* extract on glucose tolerance

There were significant differences in the area under the curve (AUC) values between the four experimental groups on Day 28 [1040 ± 32.77 mmol/L.min (negative control) vs. 827.7 ± 18.2 mmol/L.min (low dose test) vs. 880.7 ± 24.11 mmol/L.min (high dose test) vs. 827.1 ± 15.15 mmol/L.min (positive control): F (3,36) = 1.197: p = 0.0424]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences were between the negative control and low dose (50mg) ($p < 0.0001$), negative control and high dose test (100mg) ($p = 0.0002$) and negative control and positive control ($p < 0.0001$).

There were significant differences in AUC between the four experimental groups on Day 56 [1626 ± 57.06 mmol/L.min (negative control) vs. 750.5 ± 24.11 mmol/L.min (low dose test) vs. 861.3 ± 15.07 mmol/L.min (high dose test) vs. 801.8 ± 12.89 mmol/L.min (positive control): F (3,36) = 3.395: p = 0.0281]. Post-hoc statistical analysis using Tukey's multiple comparisons test showed significant differences were between the negative control and low dose test (50mg) ($p < 0.0001$), negative control and high dose test (100mg) ($p < 0.0001$) and negative control and positive control ($p < 0.0001$). The results of OGTT performed on Day 28 and week Day 56 are shown below in Figure 4.4 and the calculated mean area under the curve (AUC) values are shown in Figure 4.5.


Oral glucose tolerance: Day 28
Glucose levels(mmol/l)
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
0
30
60
90
120
Time (Minutes)
Negative Control
Low Dose test (50mg)
High dose test (100mg)
Positive Control

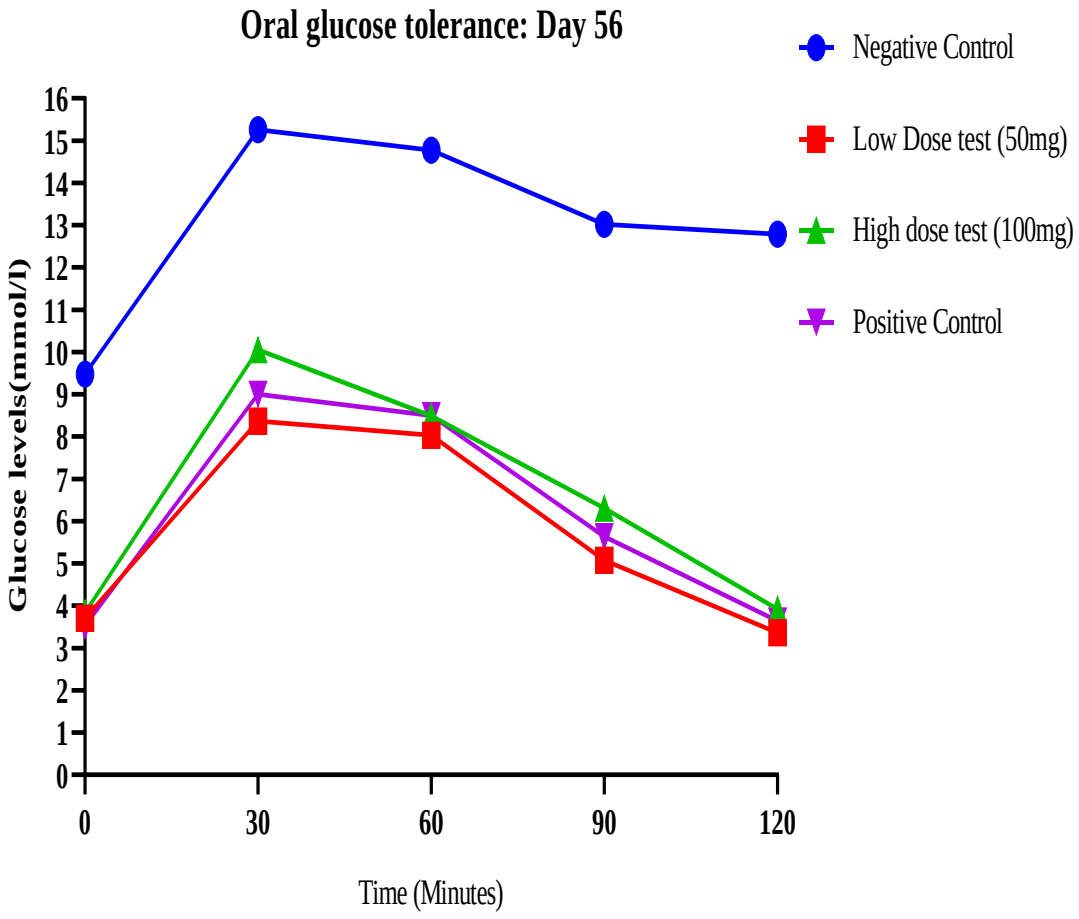


Figure 4.3: Line graphs showing fasting blood glucose levels (mmol/L) at weekly intervals during the experimental period.

Expressed as mean ± SEM. (*- $p < 0.05$, **- $p < 0.01$, ***- $p < 0.001$, ****- $p < 0.0001$).

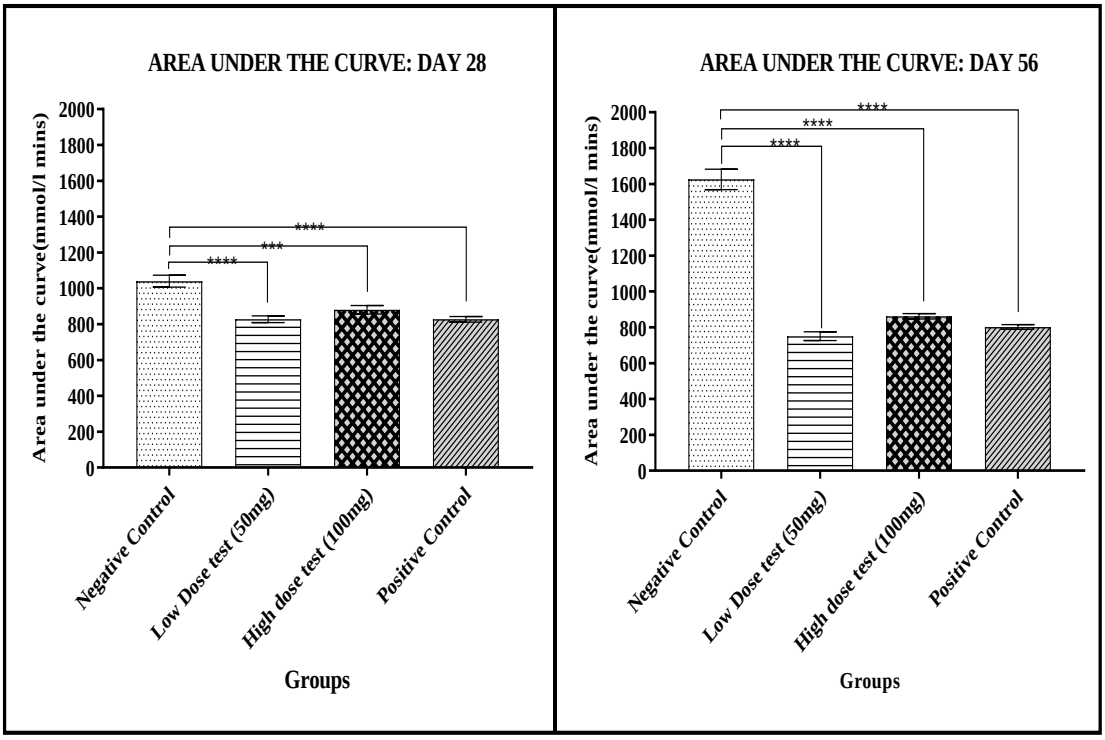


Figure 4.4: Graphs showing the mean area under the curve (mmol/L) during the oral glucose tolerance tests.

Results are expressed as mean ± SEM. ***- $p < 0.001$, ****- $p < 0.0001$.

4.5 Effect of the *Rotheca myricoides* extract on fasting insulin levels.

There were significant differences in fasting insulin levels between the four experimental groups: [1.84 ± 0.19 mU/L (negative control) vs. (0.69 ± 0.13 mU/L (low dose test) vs. (0.83 ± 0.17 mU/L (high dose test) vs. (0.69 ± 0.10 mU/L (positive control): F (3, 36) = 0.6421: $p < 0.0001$]. Post -hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the negative control and low dose test (50mg) ($p < 0.0001$), the negative control and high dose test (100mg) ($p < 0.0001$) and the negative control and positive control ($p < 0.0001$) groups. The graphical representations of these results are shown in Figure 4.5.

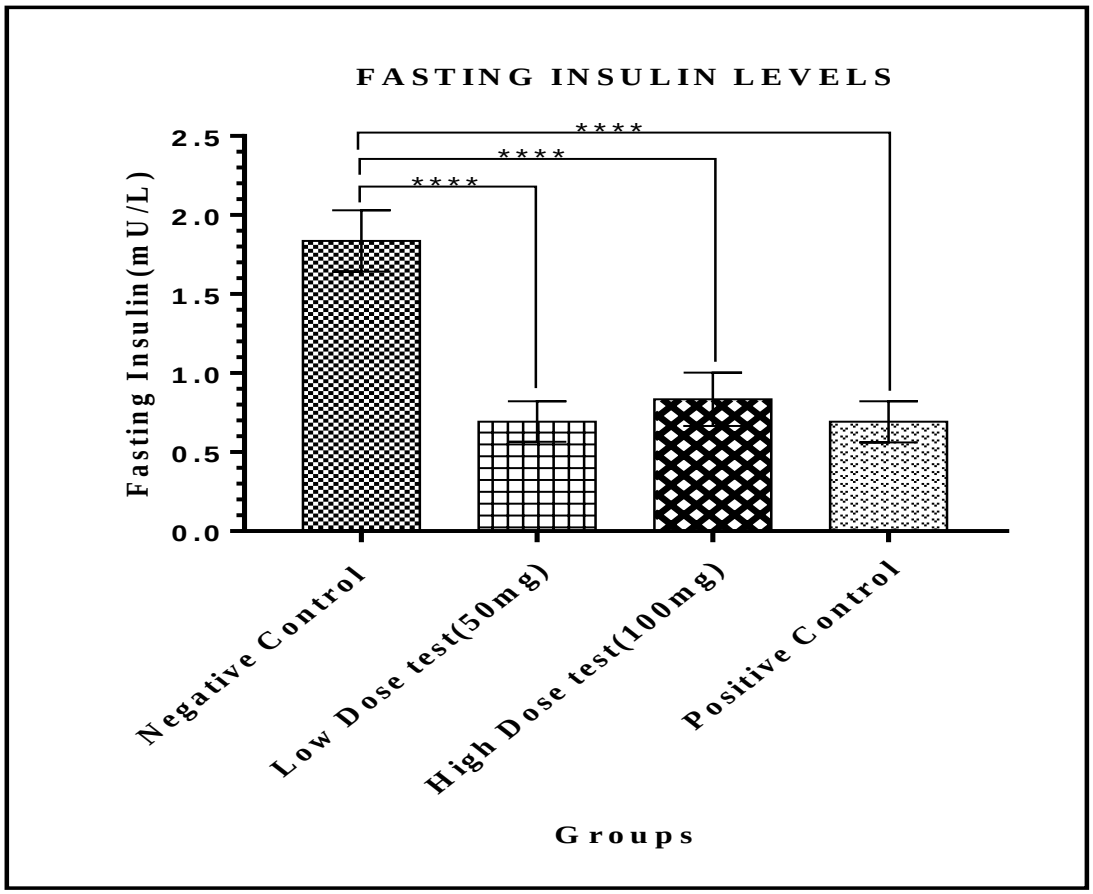


Figure 4.5: Graph showing the fasting insulin levels (mU/L) of the experimental groups.

Results are expressed as mean ± SEM. (****- $p < 0.0001$).

4.6 Outcome of *Rotheca* myricoides extract on the homeostatic model assessment of insulin resistance (HOMA-IR)

There were significant differences in HOMA-IR between the experimental four groups: [3.0 ± 0.10 (negative control) vs. (0.76 ± 0.89 (low dose test) vs. (0.89 ± 0.67 (high dose test) vs. (0.62 ± 0.08 (positive control): $F(3, 36) = 0.8041$: $p < 0.0001$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the negative control and low dose test (50mg) ($p < 0.0001$), the negative control and high dose test (100mg) ($p < 0.0001$) and the negative control and positive control ($p < 0.0001$) groups. The graphical representations of these results are shown in Figure 4.6.

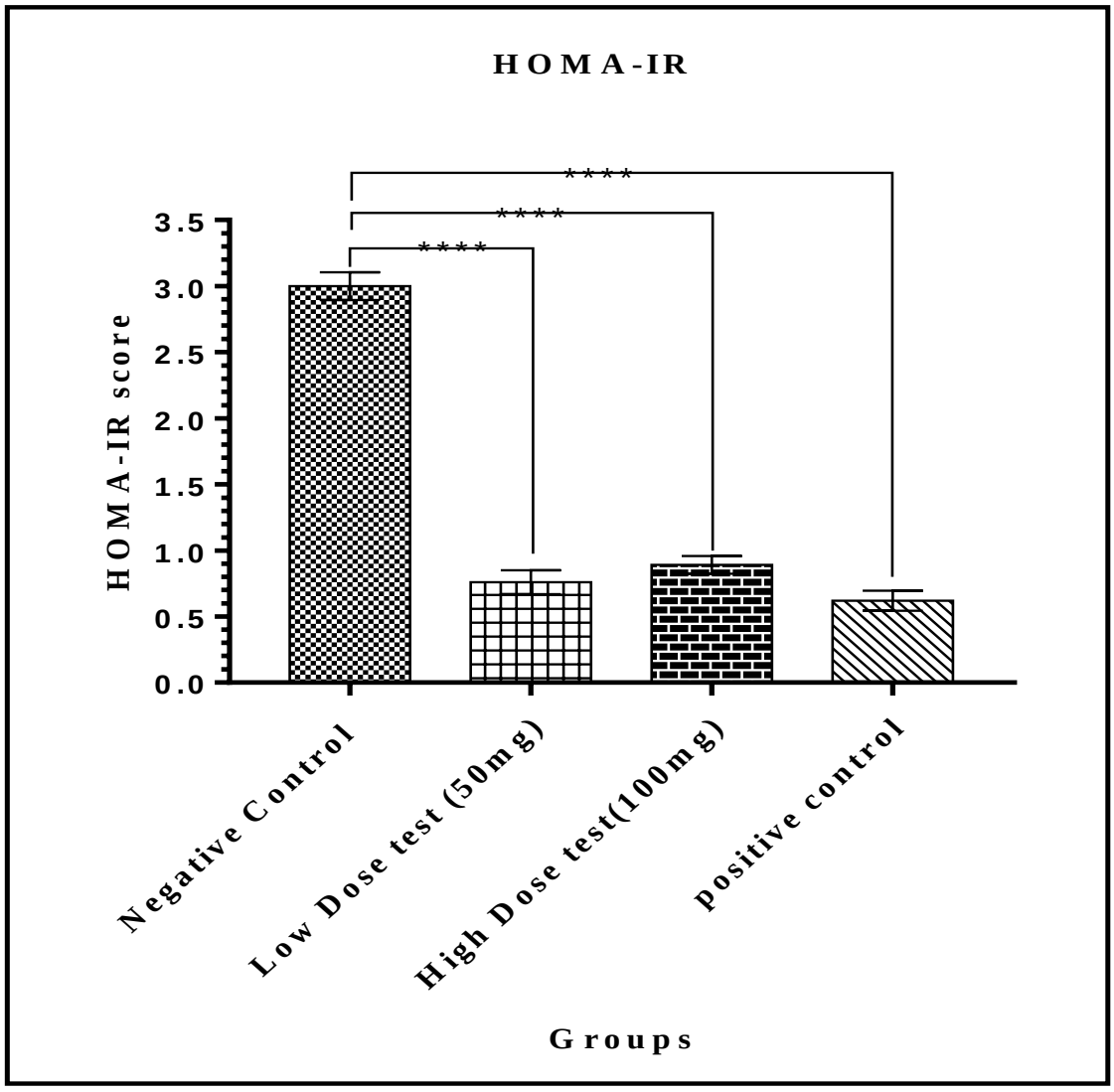


Figure 4.6: Graph showing HOMA-IR score (mU/L) of the experimental groups.

Results are expressed as mean ± SEM. (****- $p < 0.0001$).

4.7 End result of *Rotheca myricoides* extract on hepatic triglycerides.

There were significant differences in hepatic triglycerides content between the four experimental groups. [5.09 ± 0.15 mg/g (negative control) vs. 2.14 ± 0.10 mg/g (low dose) vs. 2.3 ± 0.12 mg/g (high dose) vs. 1.94 ± 0.20 mg/g (positive control): F (3, 35) = 0.7839: p < 0.0001]. Post -hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the negative control and low dose (50mg) (p < 0.0001), the negative control and high dose (100mg) (p < 0.0001) and the negative control and positive control (p < 0.0001). The graphical representations of these results are shown in Figure 4.7.

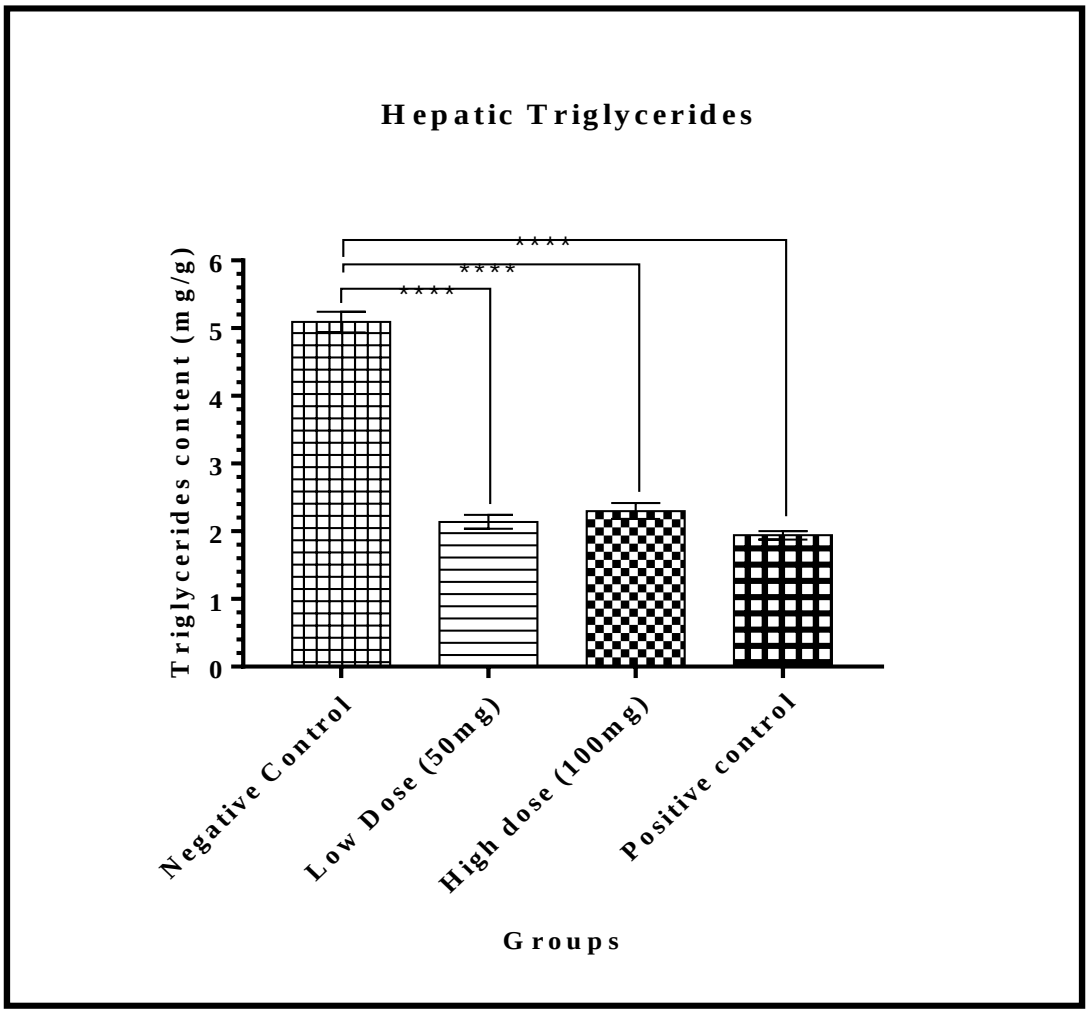


Figure 4.7: Graph depicting the mean hepatic triglycerides content (mg/g) of the experimental groups.

Results are expressed as mean ± SEM. (****- $p < 0.0001$).

4.8 Effect of *Rotheca myricoides* extract on serum lipids

There were significant differences in total plasma cholesterol between the groups [5.64 ± 0.11 mmol/L (negative control) vs. 1.62 ± 0.14 mmol/L (low dose) vs. 1.68 ± 0.11 mmol/L (high dose) vs. 1.66 ± 0.99 mmol/L (positive control): $F (3, 36) = 0.3513$: $p < 0.0001$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the negative control and low dose (50mg) ($p < 0.0001$), the negative control and high dose (100mg) ($p < 0.0001$) and the negative control and positive control ($p < 0.0001$). The graphical representations of these results are shown in Figure 4.8.

There were significant differences in total serum triglycerides between the four experimental groups [3.83 ± 0.22 mmol/L (negative control) vs. 0.60 ± 0.07 mmol/L (low dose) vs. 0.67 ± 0.10 mmol/L (high dose) vs. 0.69 ± 0.09 mmol/L (positive control): $F (3, 36) = 8.39$: $p < 0.0001$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences were between the negative control and low dose (50mg) ($p < 0.0001$), the negative control and high dose (100mg) ($p < 0.0001$) and the negative control and positive control ($p < 0.0001$). The graphical representations of these results are shown in Figure 4.9.

There were significant differences in HDL cholesterol between the groups [0.78 ± 0.08 mmol/L (negative control) vs. 1.66 ± 0.12 mmol/L (low dose) vs. 1.70 ± 0.08 mmol/L (high dose) vs. 1.69 ± 0.15 mmol/L (positive control): $F (3, 36) = 0.8401$: $p < 0.0001$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the negative control and low dose (50mg) ($p < 0.0001$), the negative control and high dose (100mg) ($p < 0.0001$) and the negative control and positive control ($p < 0.0001$). The graphical representations of these results are shown in Figure 4.10.

There were significant differences in LDL cholesterol between the four experimental groups [3.52 ± 0.19 mmol/L (negative control) vs. 0.33 ± 0.14 mmol/L (low dose) vs. 0.34 ± 0.20 mmol/L (high dose) vs. 0.33 ± 0.01 mmol/L (positive control): F (3, 36) = 13.63: $p < 0.0001$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences were between the negative control and low dose (50mg) ($p < 0.0001$), the negative control and high dose (100mg) ($p < 0.0001$) and the negative control and positive control ($p < 0.0001$). The graphical representations of these results are shown in Figure 4.11.

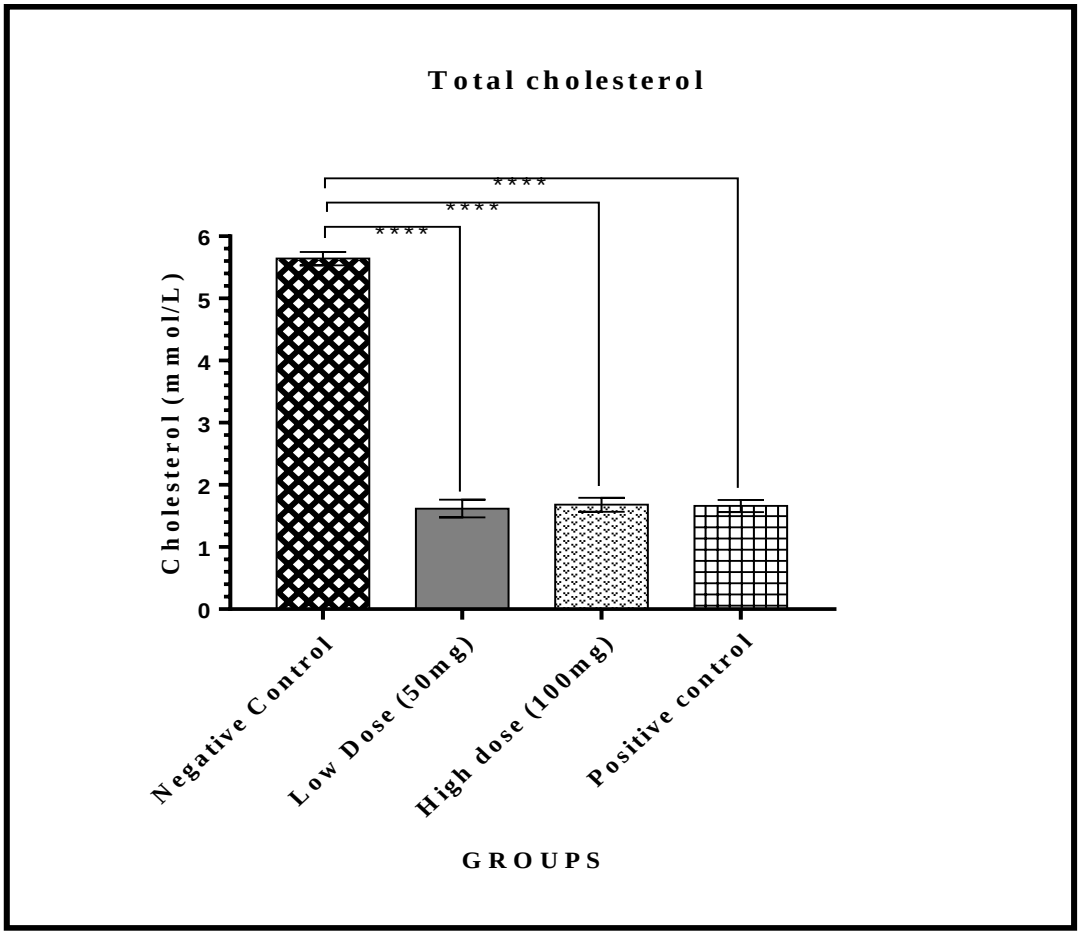


Figure 4.8: Graph showing the mean total cholesterol (mmol/L).
Results are expressed as mean ± SEM. (****- $p < 0.0001$).

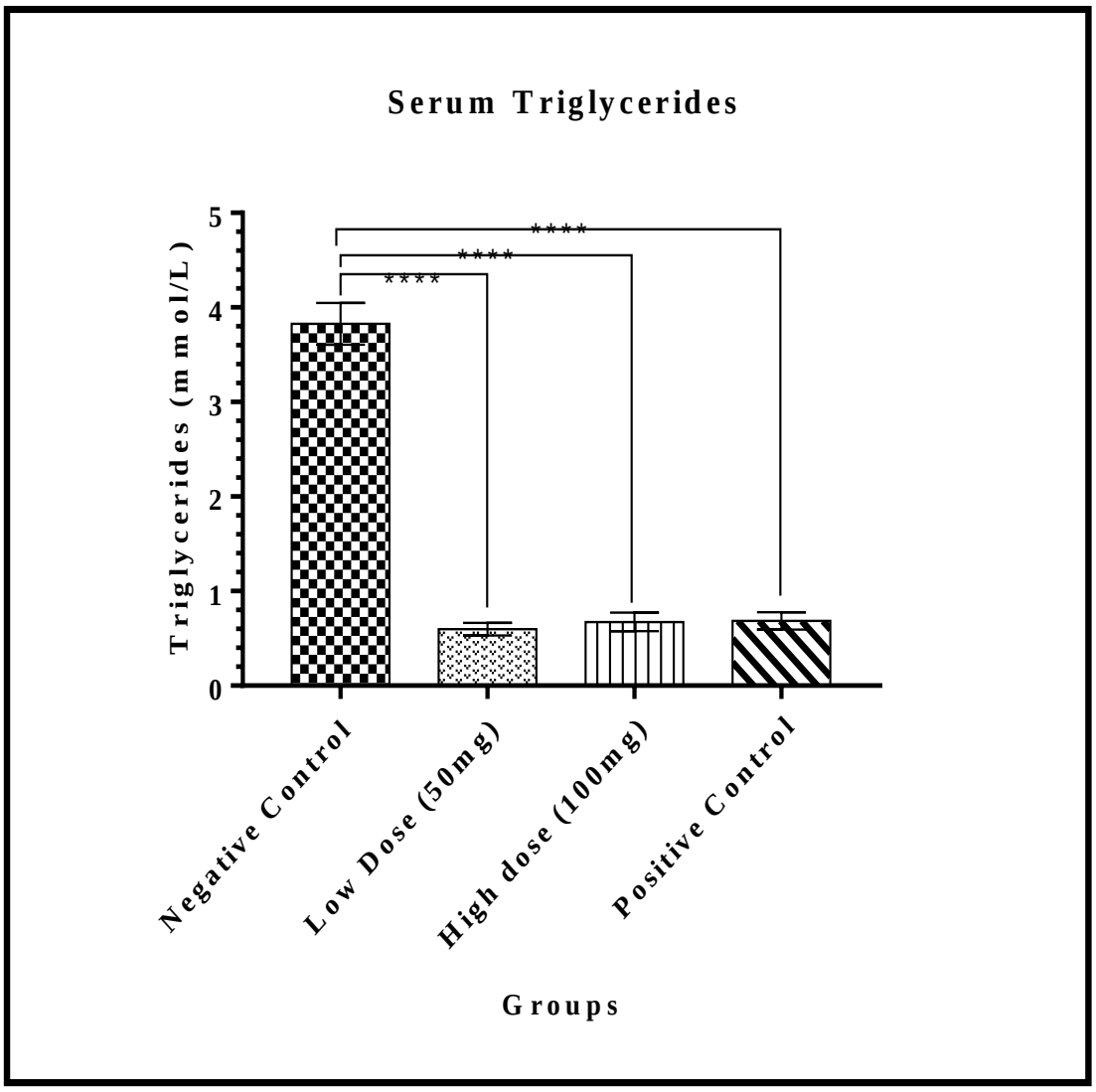


Figure 4.9: Graph showing the mean plasma triglycerides (mmol/L).

Results expressed as mean ± SEM. (****- $p < 0.0001$).

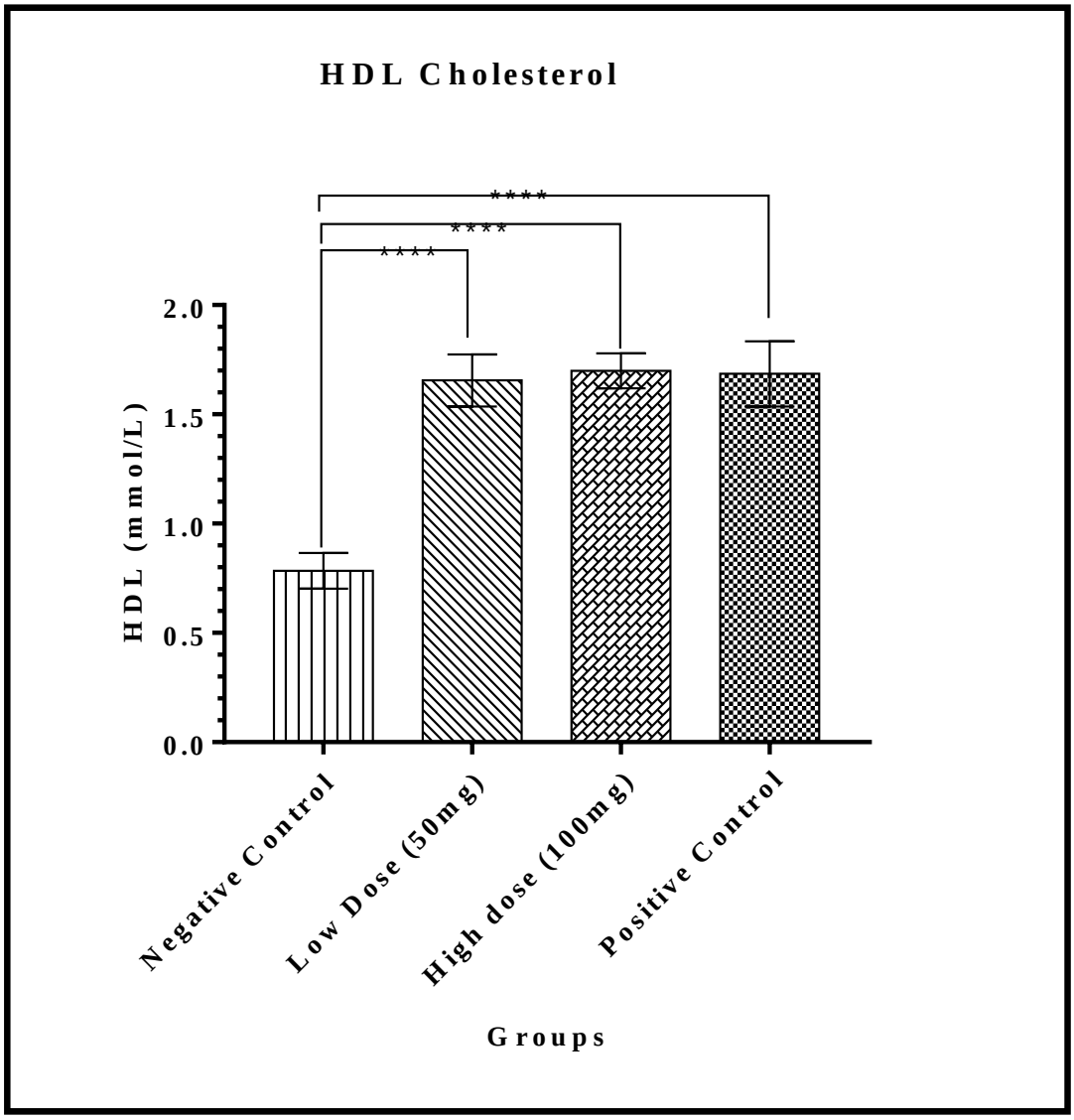


Figure 4.10: Graph showing the mean HDL cholesterol (mmol/L).

Results expressed as mean ± SEM. (****- $p < 0.0001$).

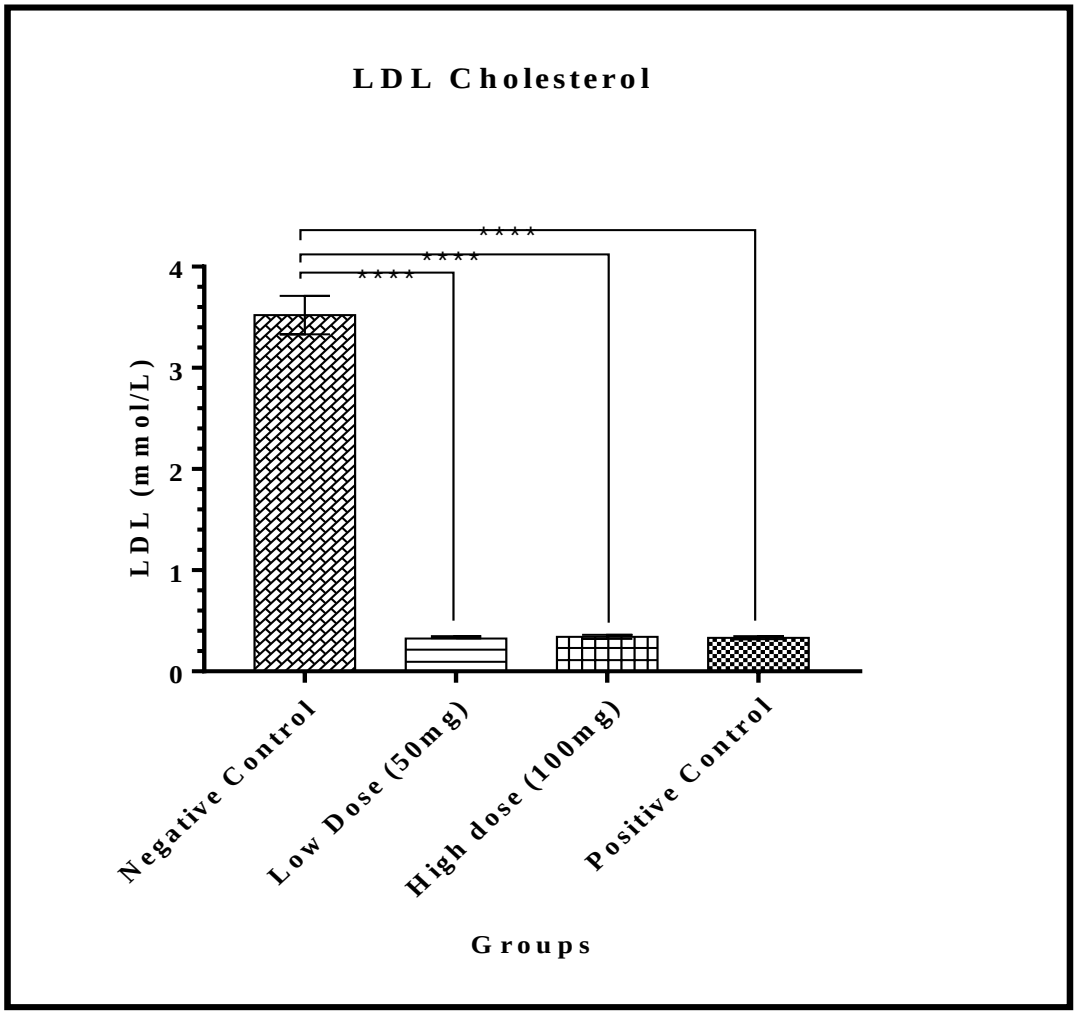


Figure 4.11: Graph showing the mean LDL cholesterol (mmol/L).

Results expressed as mean ± SEM. (****- $p < 0.0001$).

4.9 Changes on liver weight after administration of *Rotheca myricoides* extract

There were significant differences in mean liver weight between the four experimental groups [8.55 ± 0.21g (negative control) vs. (3.48 ± 0.14 g (low dose test) vs. (3.64 ± 0.10 g (high dose test) vs. (3.31 ± 0.09 g (positive control): F (3, 36) = 4.28: $p < 0.0001$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences were between the negative control and low dose test (50mg) ($p < 0.0001$), the negative control and high dose test (100mg) ($p < 0.0001$) and the negative control and positive control ($p < 0.0001$). The graphical representations of these results are shown in Figure 4.12.

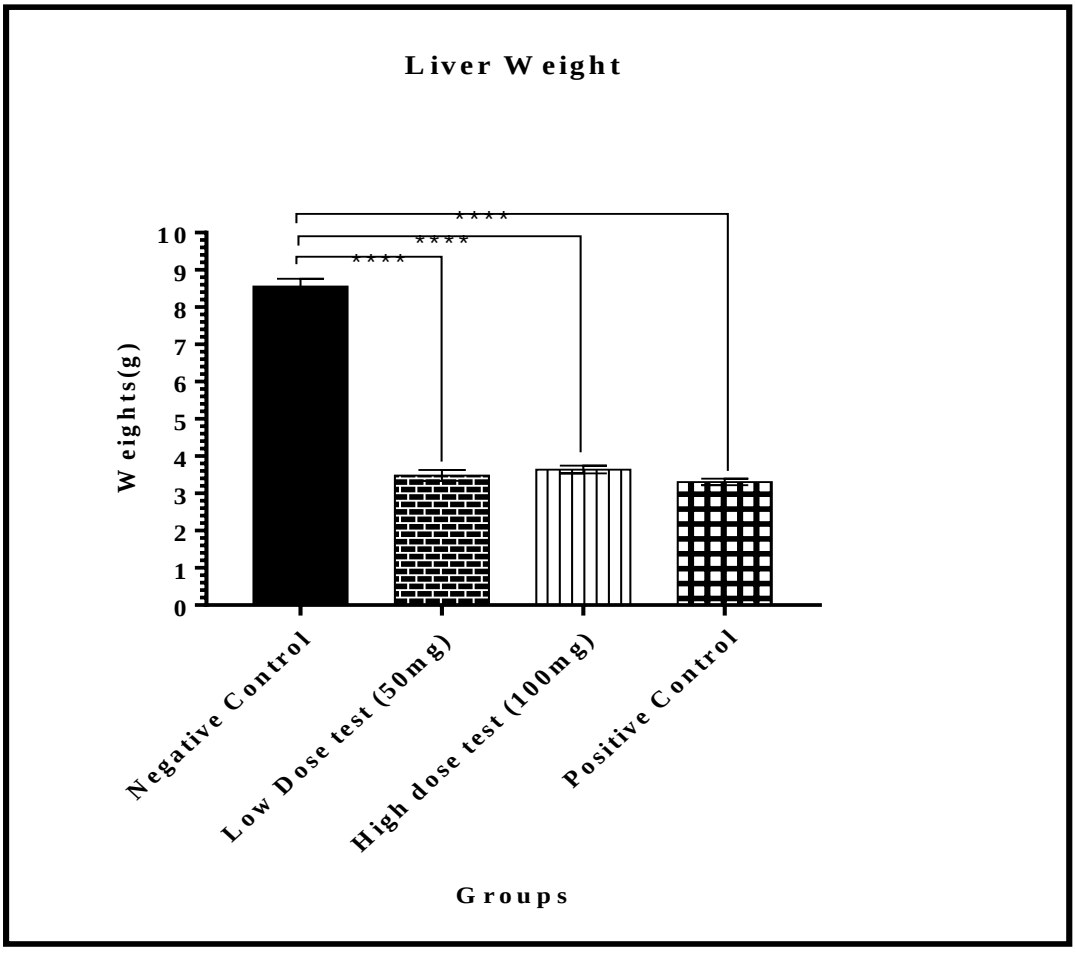


Figure 4.12: Graph depicting the mean liver weight (g).

Results expressed as mean ± SEM. (****- $p < 0.0001$).

4.10 Liver weight: body weight ratio changes after administration of *Rotheca myricoides* extract.

There were significant differences in mean liver weight: body weight ratio between the four experimental groups (0.0803 ± 0.00362 arbitrary units (negative control) vs. (0.03768 ± 0.00154 arbitrary units (low dose test) vs. (0.04192 ± 0.00310 arbitrary units (high dose test) vs. (0.07019 ± 0.00315 arbitrary units (PC): $F(3, 36) = 1.276$: ($p < 0.0001$). Post -hoc statistical analysis using Tukey's multiple comparisons test showed significant differences were between the negative control and low dose test (50mg) ($p < 0.0001$), the negative control and high dose test (100mg) ($p < 0.0001$), the low dose test (50mg) and positive control ($p < 0.0001$) and, high dose test and positive control ($p < 0.0001$). The graphical representations of these results are shown in Figure 4.13.

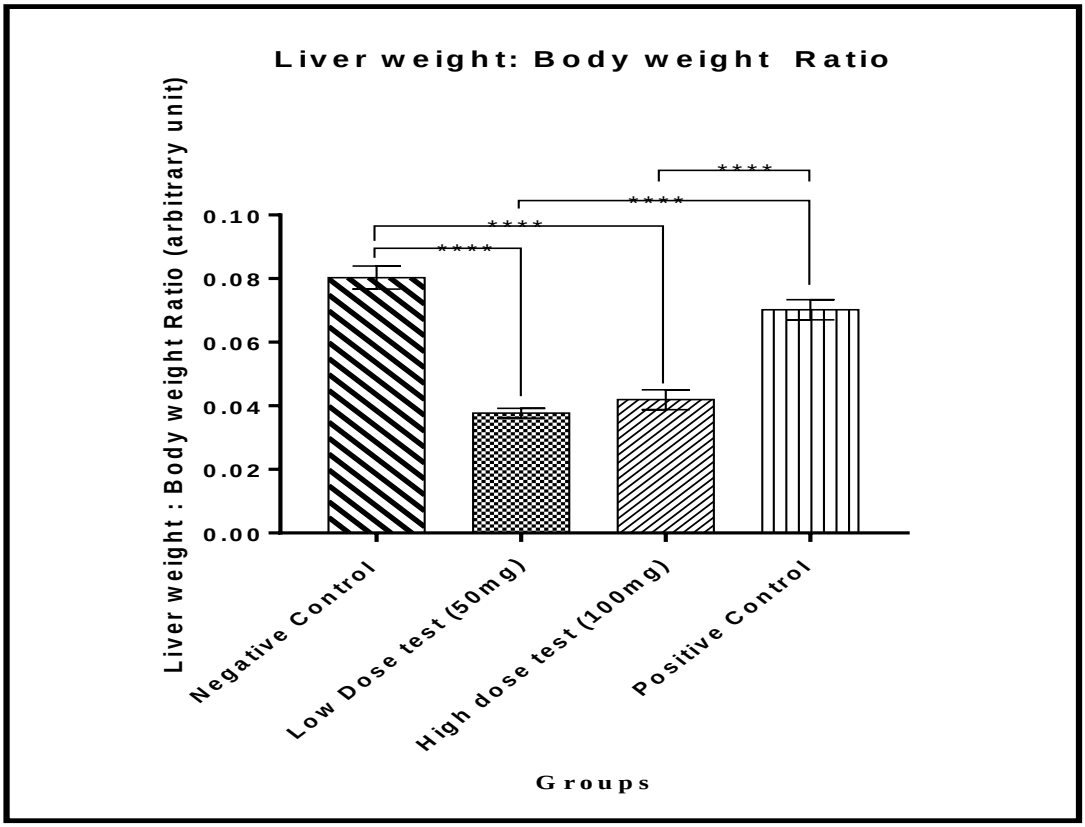


Figure 4.13: Graph showing the mean liver weight: body weight ratio (arbitrary unit).

Results expressed as mean ± SEM. (****- $p < 0.0001$).

4.11 Effect of *Rotheca myricoides* extract on serum uric acid

There were significant differences in serum uric acid between the four experimental groups [2.7 ± 0.20 mg/dL (negative control) vs. 0.33 ± 0.46 mg/dL (low dose test) vs. 0.49 ± 0.52 mg/dL (high dose test) vs. 0.48 ± 0.78 mg/dL (positive control): F (3, 36) = 5.64: $p < 0.0001$]. Post -hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences were between the negative control and low dose (50mg) ($p < 0.0001$), the negative control and high dose (100mg) ($p < 0.0001$) and the negative control and positive control ($p < 0.0001$). The graphical representations of these results are shown in Figure 4.14.

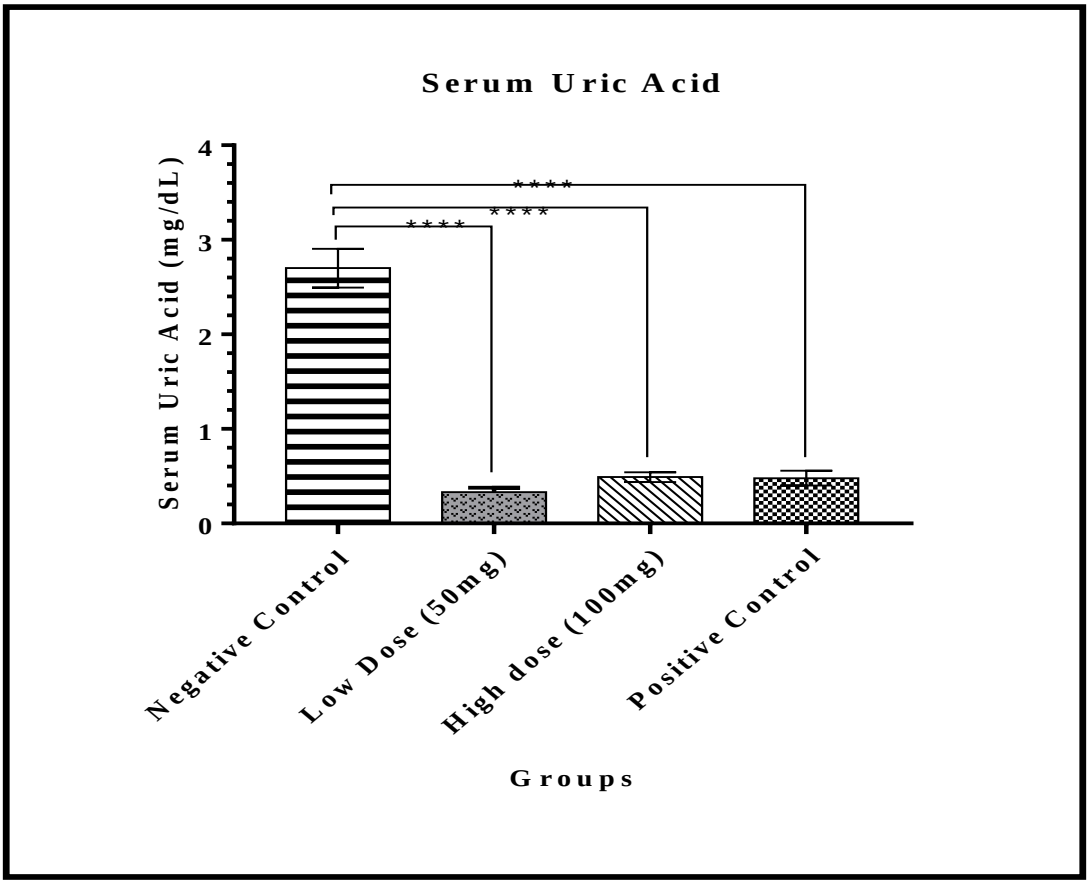


Figure 4.14: Graph showing the mean serum uric acid (mg/dL).

Results expressed as mean ± SEM. (****- $p < 0.0001$).

4.12 Outcome of *Rotheca myricoides* on retroperitoneal adipose tissue

There were significant differences in retroperitoneal adipose tissue weight between the groups [8.05 ± 0.31 grams (negative control) vs. 3.69 ± 0.32 grams (low dose) vs. 3.83 ± 0.35 grams (high dose) vs. 9.26 ± 0.53 grams (positive control): F (3, 36) = 1.796: p < 0.0001]. Post -hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the negative control and low dose (50mg) (p < 0.0001), the negative control and high dose (100mg) (p < 0.0001), low dose (50mg) and positive control (p < 0.0001) and high dose (100mg) and positive control (p < 0.0001). The graphical representations of these results are shown in Figure 4.15.

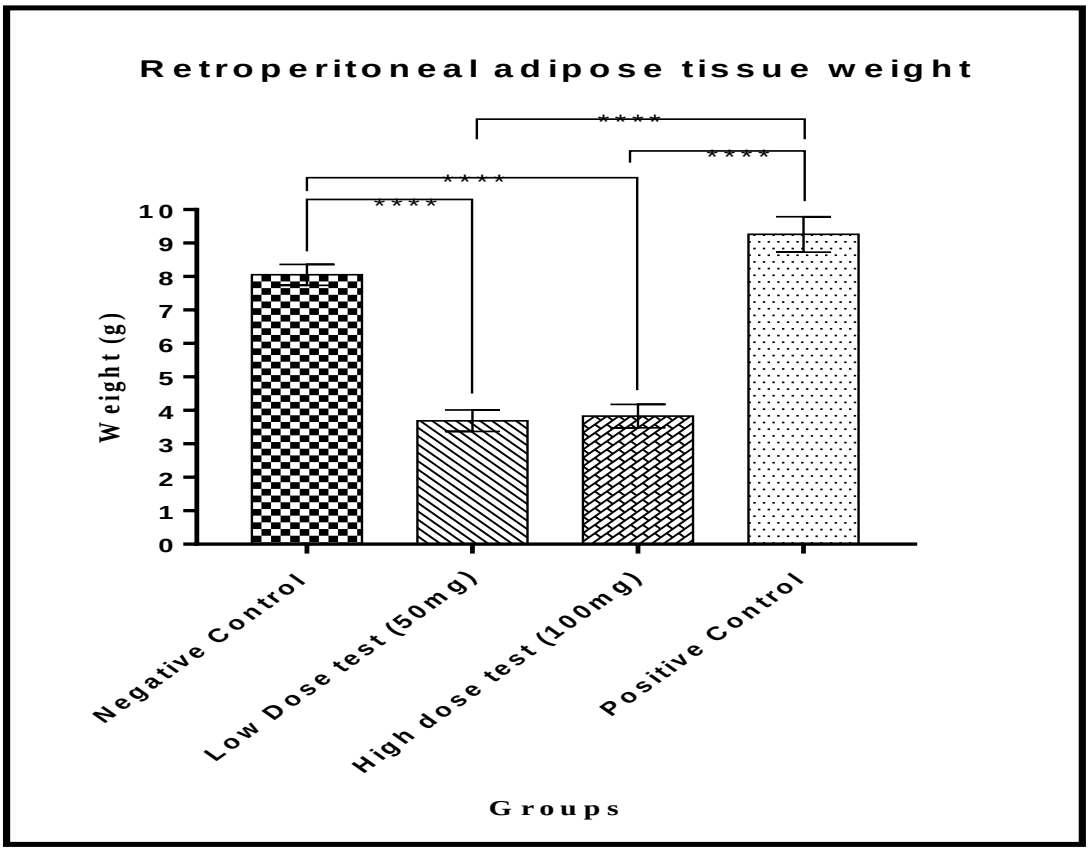


Figure 4.15: Graph showing retroperitoneal adipose tissue weight (g).

Results expressed as mean ± SEM. (****- p < 0.0001).

4.13 Outcome of *Rotheca myricoides* extract on pericardial adipose tissue

There were significant differences in pericardial adipose tissue weight between the groups [2.79 ± 0.18 grams (negative control) vs. 0.99 ± 0.14 grams (low dose) vs. 0.82 ± 0.10 grams (high dose) vs. 2.68 ± 0.20 grams (positive control): F (3, 36) = 1.847: p < 0.0001]. Post -hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the negative control and low dose (50mg) (p < 0.0001), the negative control and high dose (100mg) (p < 0.0001), low dose (50mg) and positive control (p < 0.0001) and high dose (100mg) and positive control (p < 0.0001). The graphical representations of these results are shown in Figure 4.16.

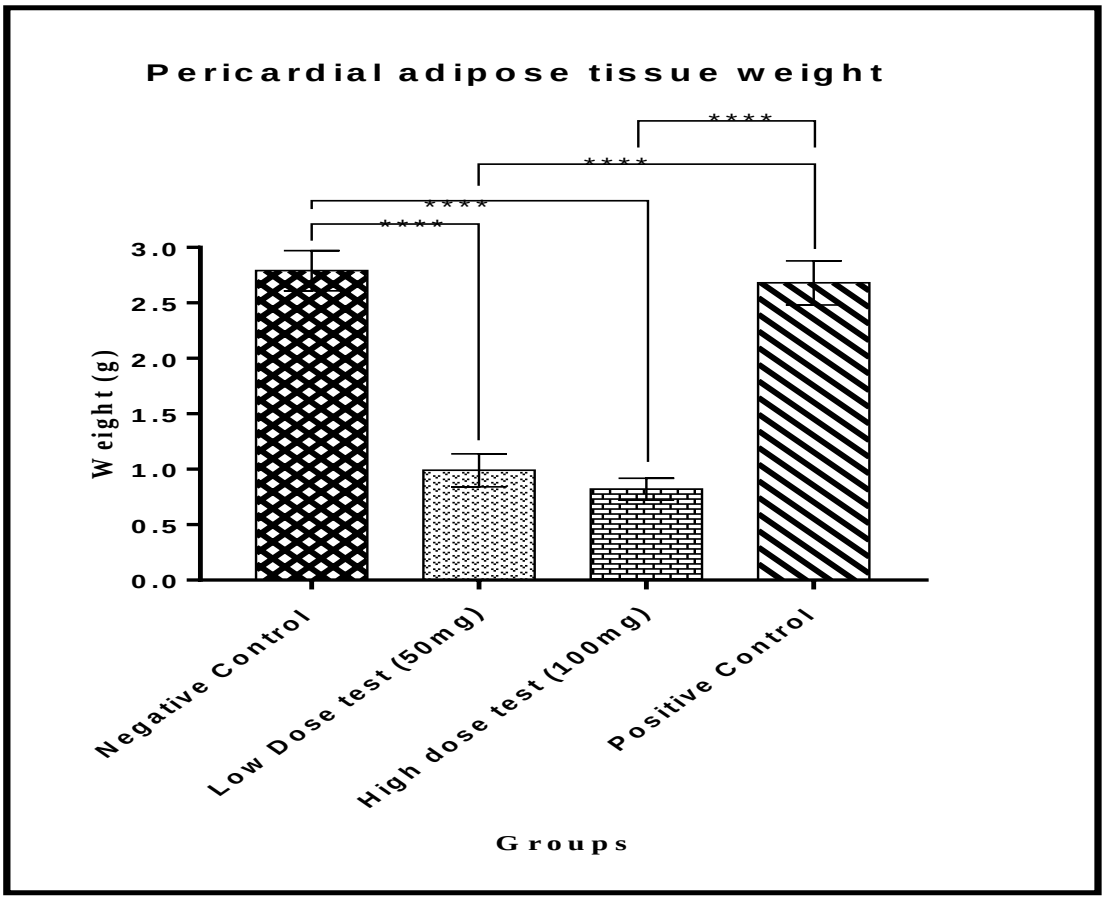


Figure 4.16: Graph showing pericardial adipose tissue weight (g).

Results expressed as mean ± SEM. (****- p < 0.0001).

4.14 Effect of the *Rotheca myricoides* extract on mesenteric adipose tissue

There were significant differences in mesenteric adipose tissue weight between the groups [12.19 ± 0.50 grams (negative control) vs. 6.62 ± 0.24 grams (low dose) vs. 6.48 ± 0.20 grams (high dose) vs. 13.36 ± 0.71grams (positive control): F (3, 36) = 4.043: $p < 0.0001$]. Post -hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the negative control and low dose (50mg) ($p < 0.0001$), the negative control and high dose (100mg) ($p < 0.0001$), low dose (50mg) and positive control ($p < 0.0001$) and high dose (100mg) and positive control ($p < 0.0001$). The graphical representations of these results are shown in Figure 4.17.

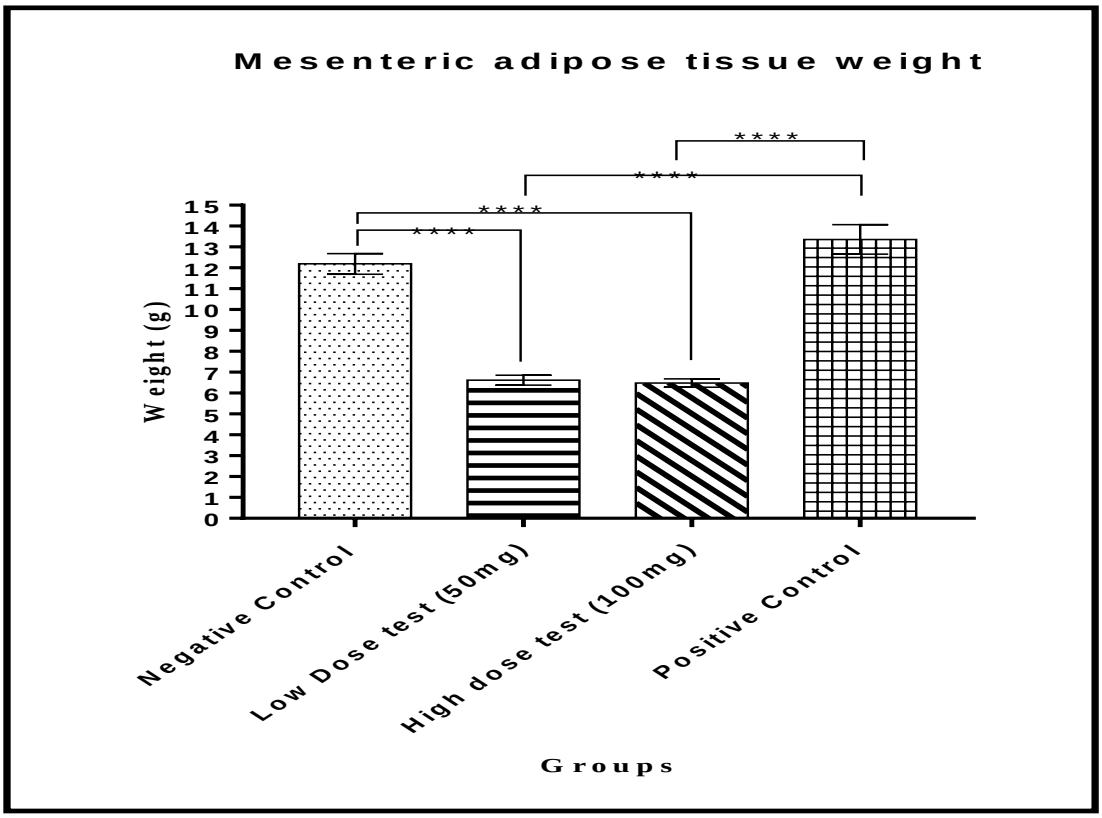


Figure 4.17: Graph showing mesenteric adipose tissue weight (g).

Results expressed as mean ± SEM. (****- $p < 0.0001$).

4.15 Mechanism of action of the *Rotheca myricoides* extract by using a Glut-4 blocker Indinavir sulphate

There were significant differences in area under the curve (AUC) values between the groups [704.2 ± 18.09 mmol/L. min (negative control) vs. (427.3 ± 15.97 mmol/L. min (positive control) vs. (707 ± 15.5 mmol/L. min (test group I) vs. (762 ± 16.92 mmol/L. min (test group II): $F(3, 20) = 0.292$: $p < 0.0001$].

Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the positive control (50mg) and negative control ($p < 0.0001$), positive control and test group I ($p < 0.0001$) and positive control and test group II ($p < 0.0001$).

The results of calculated mean area under the curve (AUC) values for the mechanism of action are shown in Figure 21 and the results of OGTT for the mechanism of action are shown in Figure 4.18.

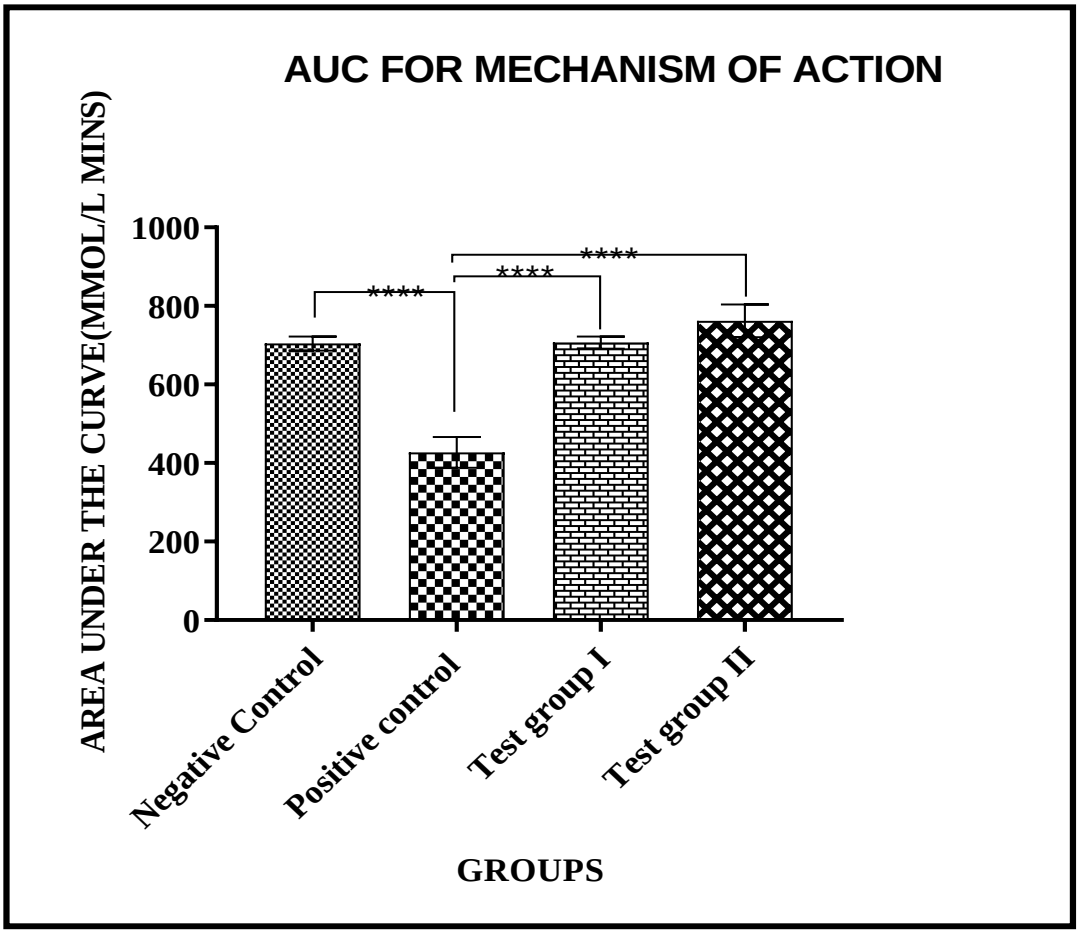


Figure 4.18: Graph showing the mean area under the curve (mmol/l) (mechanism of action of the extract).

Results are expressed as mean ± SEM. (***- $p < 0.001$, ****- $p < 0.0001$)

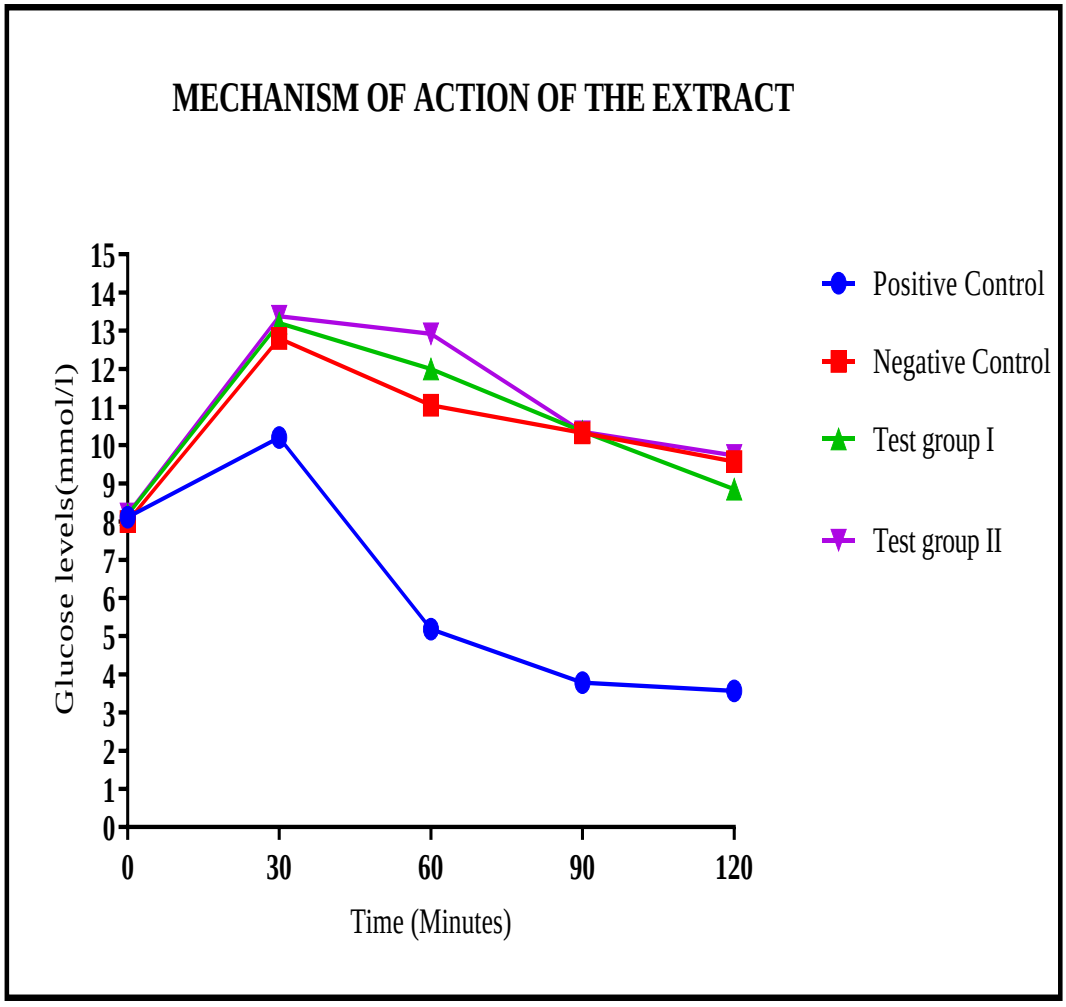


Figure 4.19: Line graph showing the mean blood glucose response (mmol/L) to an oral glucose bolus 2 g/kg over a 2-hour period, during the investigation of the mechanism of action of the extract.

4.16 Acute response of the *Rotheca myricoides* extract after oral glucose tolerance test.

There were significant differences in the acute response of the extract (50mg) [8.61 ± 0.23 mmol/L.min (0 minutes) vs. 10.26 ± 0.27 mmol/L.min (30 minutes) vs. 6.08 ± 0.27 mmol/L.min (60 minutes) vs. 3.77 ± 0.16 mmol/L.min (90 minutes) vs. 3.49 ± 0.34 mmol/L.min (120 minutes) :$F(4,45) = 2.846 : p < 0.0001$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between 0 minutes and 30 minutes ($p < 0.0001$), 0 minutes and 60minutes ($p < 0.0001$), 0 minutes and 90 minutes ($p < 0.0001$), 0 minutes and 120 minutes ($p < 0.0001$), 30 minutes and 60 minutes ($p < 0.0001$), 30 minutes and 90 minutes ($p < 0.0001$), 30 minutes and 120 minutes ($p < 0.0001$), 60 minutes and 90 minutes ($p < 0.0001$), 60 minutes and 120 minutes ($p < 0.0001$). The graphical representations of these results are shown in Figure 4.20.

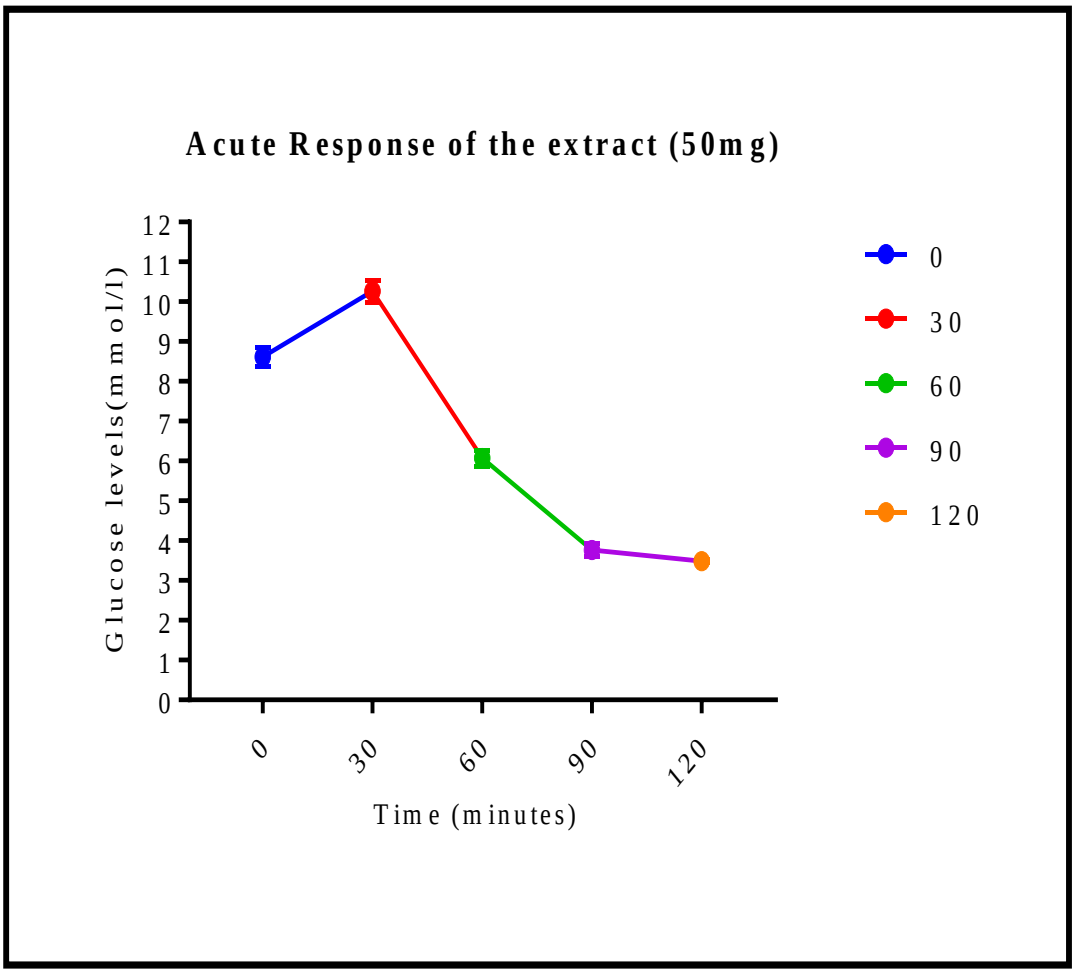


Figure 4.20: Line graph showing the mean blood glucose response (mmol/L) to an oral glucose bolus 2 g/kg over a 2-hour period, during the investigation of the acute response of the extract.

4.17 Effect on *Rotheca myricoides* extract on the branched chain amino Raman spectra.

There were statistically significant differences in the mean Raman intensity of leucine between the four experimental groups [0.8302 ± 0.02017 arbitrary units (negative control) vs. 0.3465 ± 0.03014 arbitrary units (low dose) vs. (0.3025 ± 0.032 arbitrary units (high dose) vs. 0.3168 ± 0.02795 arbitrary units (positive control): F (3, 20) = 0.4811: $p < 0.0001$]. Post-hoc statistical analysis using Tukey's multiple comparisons test showed significant differences were between the negative control and low dose (50mg) ($p < 0.0001$), the negative control and high dose (100mg) ($p < 0.0001$), the negative control and positive control ($p < 0.0001$). The graphical representations of these results are shown in Figure 4.21.

There were significant differences in the mean Raman intensity of isoleucine between the four experimental groups [0.8043 ± 0.04256 arbitrary units (negative control) vs. 0.2897 ± 0.04963 arbitrary units (low dose) vs. 0.3233 ± 0.3429 arbitrary units (high dose) vs. 0.2948 ± 0.02825 arbitrary units (positive control): F (3, 20) = 0.8578: $p < 0.0001$]. Post-hoc statistical analysis using Tukey's multiple comparisons test showed significant differences were between the negative control and low dose (50mg) ($p < 0.0001$), the negative control and high dose (100mg) ($p < 0.0001$), the negative control and positive control ($p < 0.0001$). The graphical representations of these results are shown in Figure 4.22.

There were significant differences in the mean Raman intensity of valine between the four experimental groups [0.8302 ± 0.02017 arbitrary units (negative control) vs. 0.3465 ± 0.03014 arbitrary units (low dose) vs. 0.3025 ± 0.0032 arbitrary units (high dose) vs. 0.3168 ± 0.02795 arbitrary units (positive control): F (3, 20) = 0.4811: $p < 0.0001$]. Post -hoc statistical analysis using Tukey's multiple comparisons test showed significant differences were between the negative control and low dose (50mg) ($p < 0.0001$), the negative control and high dose (100mg) ($p < 0.0001$), the negative control and positive control ($p < 0.0001$). The graphical representations of these results are shown in Figure 4.23.

There were significant differences in the mean Raman intensity of creatine monohydrate between the four experimental groups [0.677 ± 0.0483 arbitrary units (negative control) vs. 0.2695 ± 0.05983 arbitrary units (low dose) vs. 0.3365 ± 0.03915 arbitrary units (high dose) vs. 0.3483 ± 0.105 arbitrary units (positive control): F (3, 20) = 1.51: p = 0.0018]. Post-hoc statistical analysis using Tukey's multiple comparisons test showed significant differences were between the negative control and low dose (50mg) (p = 0.0021), the negative control and high dose (100mg) (p = 0.0099), the negative control and positive control (p = 0.0130). The graphical representations of these results are shown in Figure 4.24.

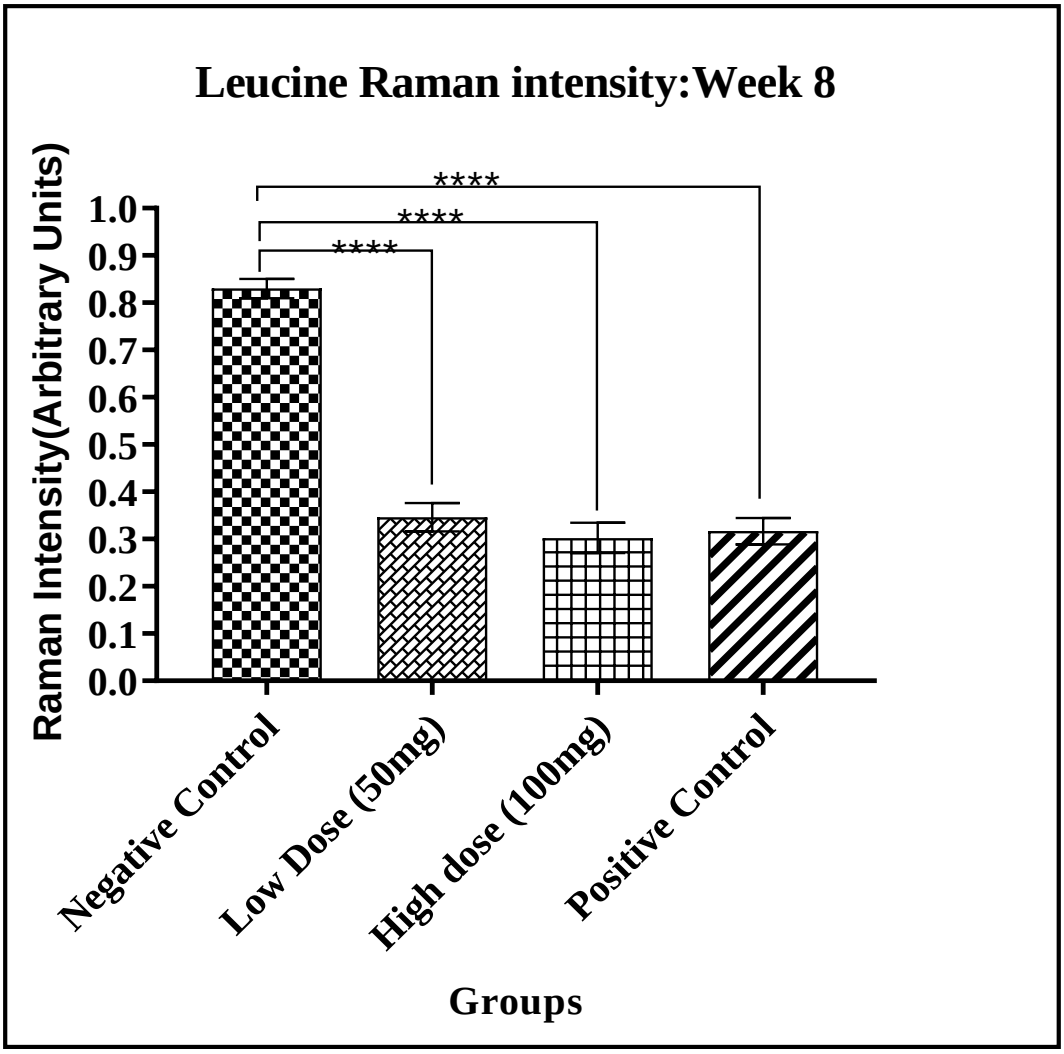


Figure 4.21: Graph depicting the mean leucine Raman intensity (arbitrary units).

Results are expressed as mean ± SEM. (****- $p < 0.0001$)

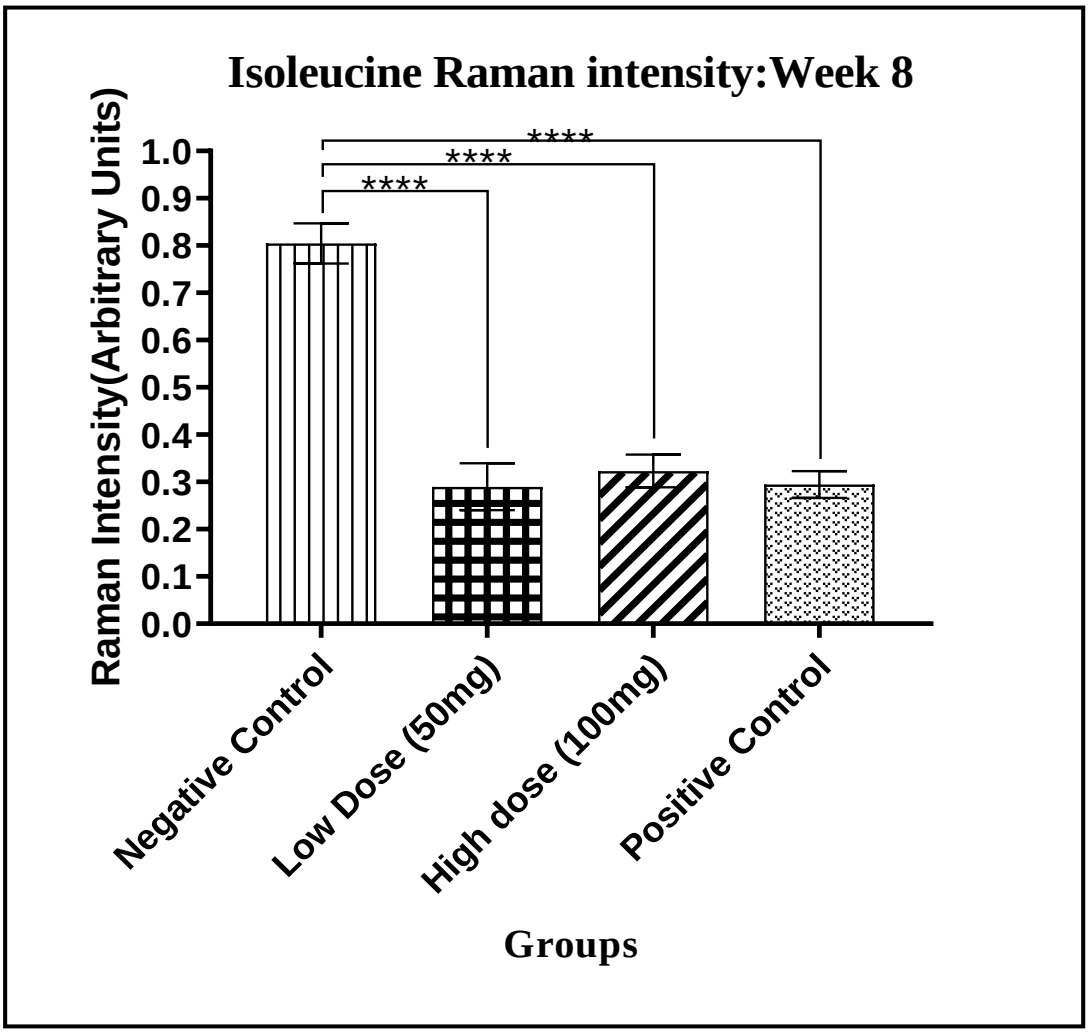


Figure 4.22: Graph depicting the mean Isoleucine Raman intensity (arbitrary units).

Results are expressed as mean ± SEM. (****- $p < 0.0001$).

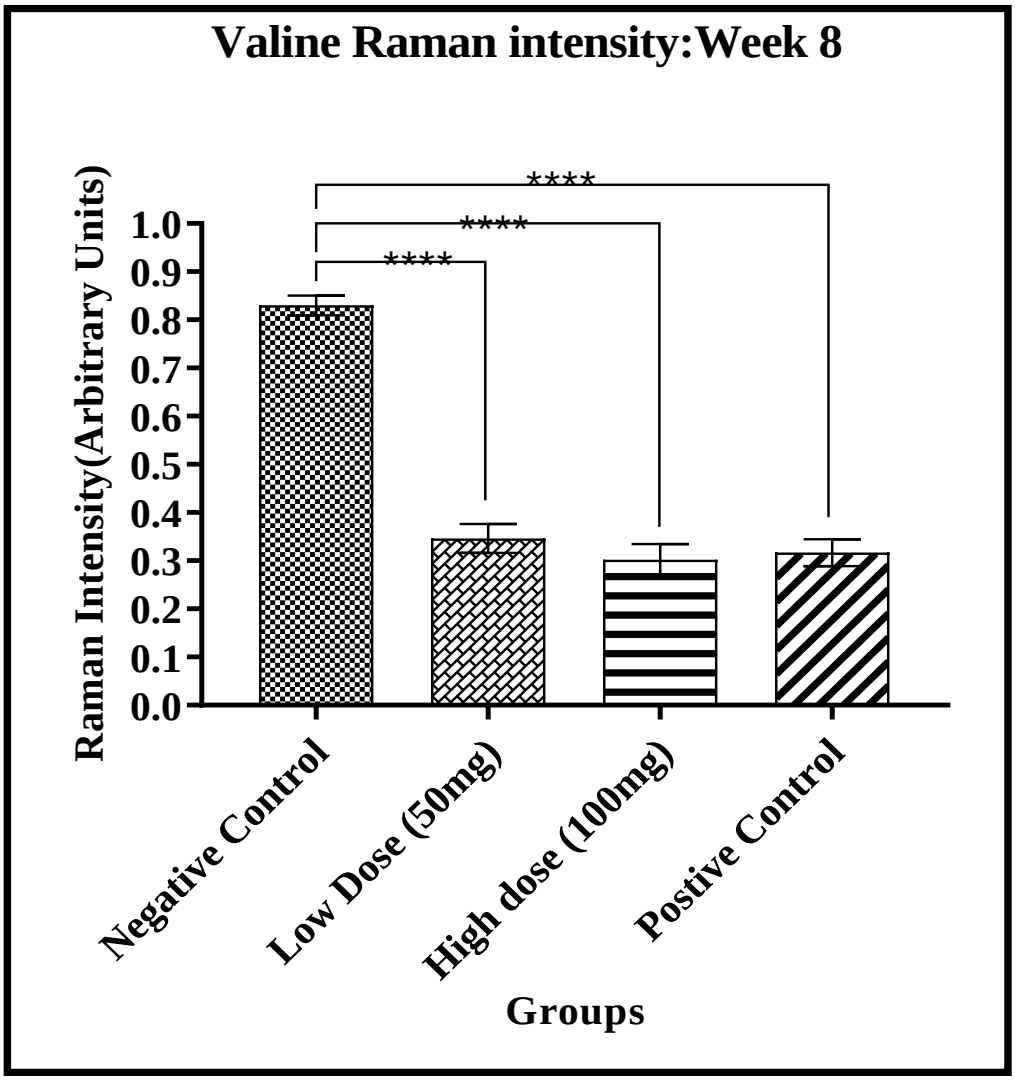


Figure 4.23: Graph depicting the mean valine Raman intensity (arbitrary units). Results are expressed as mean ± SEM. (****- $p < 0.0001$).

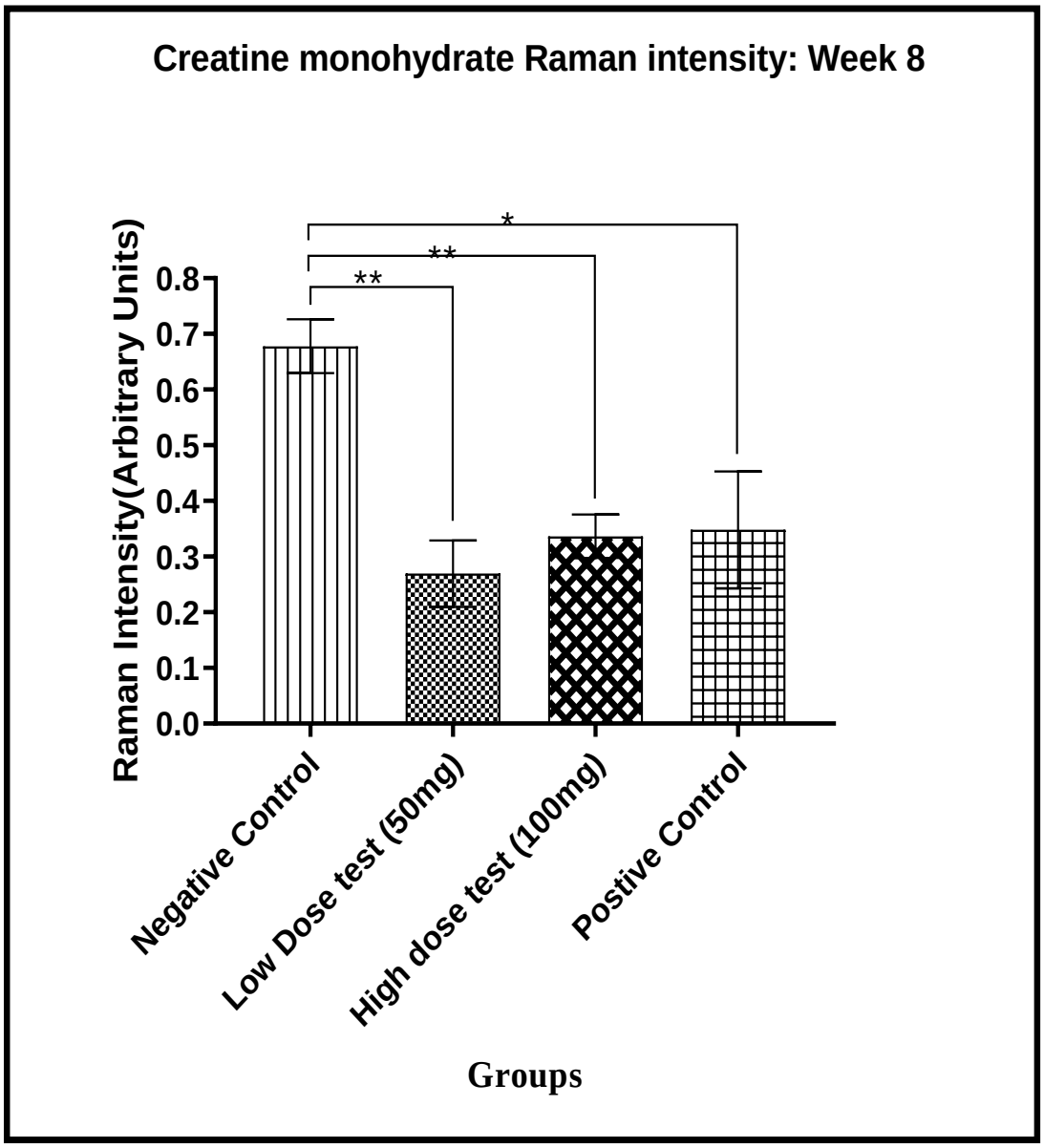


Figure 4.24: Graph showing the mean creatine monohydrate Raman intensity (arbitrary units). Results are expressed as mean ± SEM. (*- $p < 0.05$, **- $p < 0.01$).

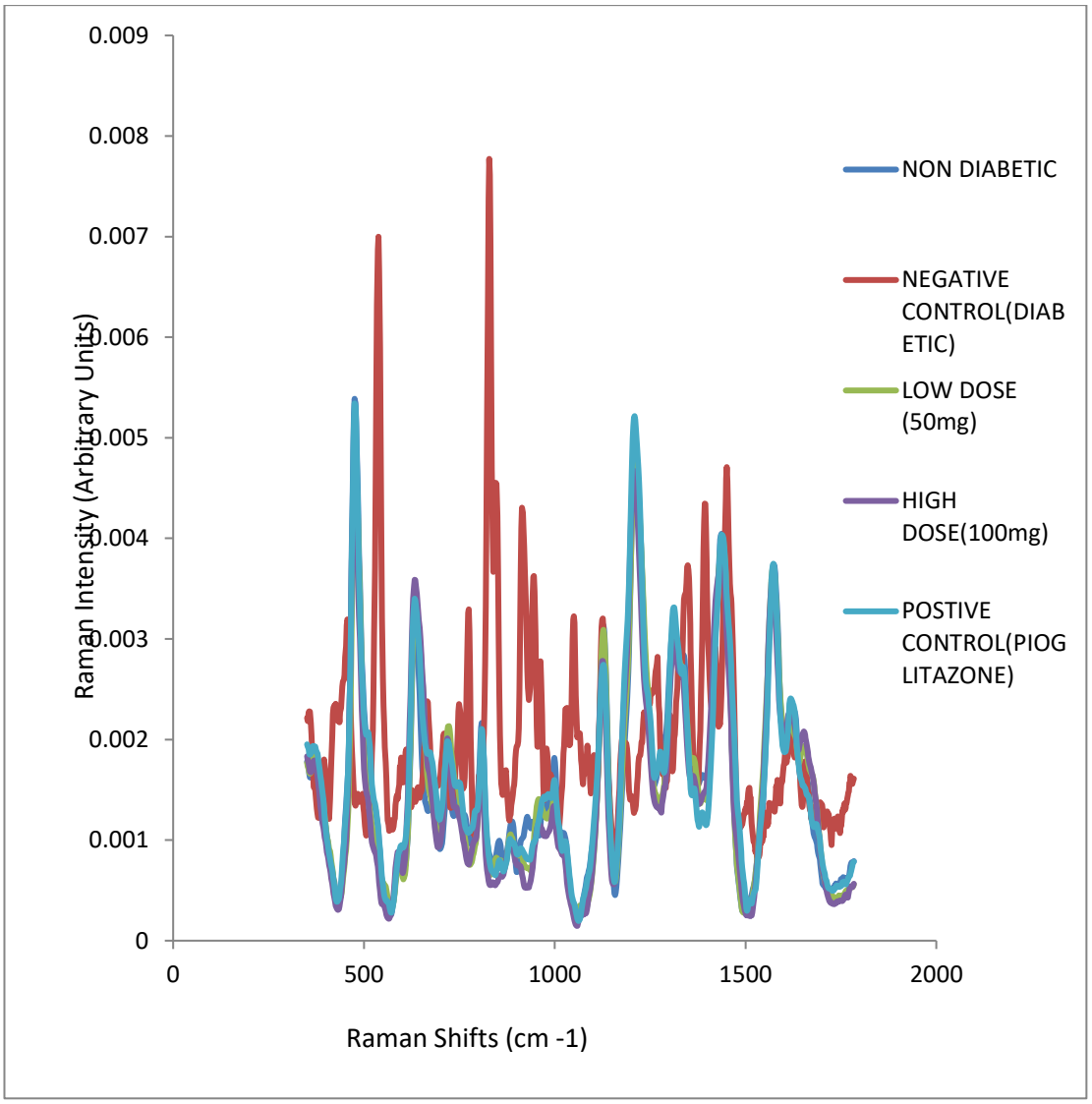


Figure 4.25: Diffractogram showing Raman spectra of blood samples at 8 weeks.

CHAPTER FIVE

5.1 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

Diabetes mellitus is a metabolic disorder frequently associated with the development of micro- and macrovascular complications that include but are not limited to: neuropathy, nephropathy, cardiovascular and cerebrovascular disease (Altan, 2003). Diabetes is therefore associated with a reduced quality of life as well as being a risk factor for increased mortality (Strojek, 2003).

Globally, the prevalence of type 2 diabetes is increasing at an alarming rate. The number of people with type 2 diabetes mellitus worldwide is projected to increase from 171 million in 2010 to 366 million by the year 2030 (Shaw *et al*., 2010).This increase, closely linked to the upsurge of obesity, represents a global health care problem. An effective strategy to restrain the epidemic increase in disease prevalence is of great importance (Masih and McIlwaine, 2009).

Current treatment modalities do not cure or reverse the progression of the condition as end organ damage still develops despite adequate glycaemic control, underscoring the need for newer more efficacious drugs for the management of diabetes mellitus (Kasper and Giovannucci, 2006). Additionally current antidiabetic drugs are often associated with toxic adverse effects (Dong *et al*., 2014). There is therefore a pressing need to develop newer safer more efficacious drugs.

Rotheca myricoides (Hochst.) Steane & Mabb belongs to the genus Rotheca comprises more than 500 species and varieties and is the largest genus of the family Verbenaceae (Bashwira and Hootele, 1988). The plant *Rotheca myricoides* (Hochst.) Steane & Mabb is used in traditional medicine for the management of diabetes (Keter and Mutiso, 2012) and this study aimed to evaluate its efficacy in an animal model of type 2 diabetes.

A high fat-high fructose diet was used for the induction of type 2 diabetes (Gan *et al*., 2014). Such a diet has previously been shown to cause the development of obesity,

hyperinsulinemia, and insulin resistance (Flanagan *et al*., 2008). On the other hand, the Initial β-cell dysfunction that mimics that observed in type 2 diabetes mellitus was achieved by the intraperitoneal administration of low dose of streptozocin. Streptozocin (STZ) has been observed to be a better chemical inducer of diabetes than alloxan (Szkudelski, 2001). This model best mimics human type 2 diabetes where obesity is associated with insulin resistance and beta cell dysfunction which ensues in the late stage of the disease (Kasper and Giovannucci, 2006).

One of the aims of the study was to create a type 2 diabetes model through a high fat-high fructose diet to achieve obesity and insulin resistance followed by a low dose of streptozocin administration to achieve type 2 diabetes. The animals in the negative control (high fat-high fructose diet group) showed greater weight gain, fasting plasma glucose levels of more than 7.0 mmol/L and impaired oral glucose tolerance of more than 11.1 mol/L compared to the other experimental groups.

The freeze-dried extracts of *Rotheca myricoides* (Hochst.) Steane & Mabb possessed significant anti-obesity in comparison to the negative control (high fat-high fructose diet group) although this effect did not appear to be dose-dependent. These results are in contrast with a previous study in which *Rotheca capitatum* did not have anti-obesity effects (A. A. Adeneye *et al*., 2008). It has been well established that chronic consumption of high fat-high fructose diet leads to dysregulation of insulin signalling which results to hyperglycaemia (Castro *et al*., 2015). Subsequent hyperglycaemia results in increased glucose-dependent insulin secretion which induces fat accumulation in adipocytes and in the liver, with consequent increase in weight which causes obesity (Perry *et al*., 2014). *Rotheca myricoides* (Hochst.) Steane & Mabb may indirectly reduce weight gain by maintaining proper insulin signalling thereby preventing hyperglycaemia and subsequent fat accumulation.

Rotheca myricoides (Hochst.) Steane & Mabb may also reduce weight gain by inhibiting an enzyme known as fatty acid synthase. This enzyme is important in fatty acid synthesis. Fatty acid synthase act by catalysing the reductive synthesis of palmitate, a long-chain fatty acid (saturated fatty acid) from malonyl-CoA and acetyl-CoA cycle in the presence of NADPH (Winzell and Ahrén, 2004). Saturated fatty acids

mediate decreased expression of PGC-1, PGC-1, and oxidative phosphorylation genes as well as impaired mitochondrial function. These effects appear to be largely mediated at a transcriptional level through by-products of fatty acid oxidation and p38 MAPK pathway activation (Fan *et al*., 2004).

The freeze-dried extracts of *Rotheca myricoides* (Hochst.) Steane & Mabb at the doses tested in this study possessed significant antihyperglycemic effects as shown in the fasting blood glucose. These antihyperglycemic effects were significantly inhibited when the extract was co-administered with indinavir which is a known GLUT4 blocker (Hruz *et al*., 2002). In addition, they also possessed significant effects on insulin sensitivity as shown in the oral glucose tolerance test. The observed effects in that study can be attributed to the enhanced glucose uptake by adipose tissue and muscle secondary to the upregulation of GLUT-4 transporters expression (Shepherd and Kahn, 1999) as well as the inhibition of hepatic glucose production and increased insulin secretion (Hui *et al*., 2009).

Oral glucose tolerance has been found to be more effective than intravenous glucose tolerance test, can be used in early diagnosis of diabetes, is less painful and easier to perform (Bartoli *et al*., 2011). Oral glucose tolerance test has been found to be a gold standard method in assessing insulin sensitivity and beta cell function, the lower the insulin sensitivity the higher the insulin concentration and the higher the insulin sensitivity, the lower the insulin concentrations so that the product of Beta-cell function and insulin sensitivity is approximately a constant (Yeckel *et al*., 2004). The results obtained in this study are similar to others in published literature. Indeed, extracts from related species, *Rotheca capitatum* possessed significant antihyperglycemic effects and improved insulin sensitivity in the oral glucose test in a dose dependent manner (A. Adeneye *et al*., 2008).

The observed effects in that study were attributed to maintenance of proper insulin signalling through the activation of Adenosine Monophosphate-activate Protein Kinase (AMPK), as has been documented for *Rotheca capitatum* (A. Adeneye *et al*., 2008). Adenosine Monophosphate-activate Protein Kinase is a cellular energy sensor that promotes glucose uptake by adipose tissue and muscle secondary to the

upregulation of GLUT-4 transporters (Shepherd and Kahn, 1999) and activates catabolic pathways which regenerate Adenosine Triphosphate (ATP) The activated AMPK catalyses the phosphorylation of Insulin Receptor Substrate-1 (IRS-1) at serine residue-789 (Ser-789), enhancing insulin-mediated tyrosine phosphorylation of IRS-1 and intracellular insulin signalling (Shepherd and Kahn, 1999) as well as the inhibition of hepatic glucose production and increased insulin secretion (Hui *et al*., 2009).

The freeze- dried extracts of *Rotheca myricoides* (Hochst.) Steane & Mabb also possessed significant effects on serum insulin levels as well on the HOMA-IR levels clearly demonstrating potent anti-hyperinsulinemic effects and/or reductions in insulin resistance. The homeostasis model assessment of Insulin Resistance (HOMA-IR) is an index that has been used to evaluate both insulin resistance and beta-cell function (Singh and Saxena, 2010). Indeed, HOMA-IR is widely accepted to be a simple and particularly helpful tool in the assessment of insulin resistance both in pre-clinical and epidemiological studies, including subjects with both glucose intolerance, mild to moderate diabetes, and in other insulin-resistance conditions (Antunes *et al*., 2016). The foregoing discussion therefore indicates that the observed antihyperglycemic effects of the extract are secondary to its insulin sensitizing effects.

The freeze-dried extracts of *Rotheca myricoides* (Hochst.) Steane & Mabb caused significant reductions in hepatic weights as well as in the hepatic triglyceride concentrations. These experimental results are similar to those in published literature. Indeed, two related plant species *Rotheca infortunatum* (Das *et al*., 2011) and *Rotheca capitatum* (A. A. Adeneye *et al*., 2008) have been shown to have similar effects. Under physiological conditions, the low steady-state triglycerides concentrations in the liver are attributable to a precise balance between acquisition by uptake of non-esterified fatty acids from the plasma and by de novo lipogenesis, versus triglycerides disposal by fatty acid oxidation and by secretion of triglycerides-rich lipoprotein (Richards *et al*., 2006). The hepatic triglyceride content test is designed to measure relative lipid accumulation in the liver which occur due to insulin resistance in type 2 diabetes (Jiménez-Agüero *et al*., 2014).

In the liver, it is believed that an increase in liver diacylglycerol (DAG) levels lead to protein kinase Cε (PKCε) activation and its consequent translocation to the cell membrane, which results in inhibition of hepatic insulin signalling. This then results in the development of hepatic insulin resistance hence hyperinsulinemia (Perry *et al*., 2014). Hyperinsulinemia induces SREBP-1c expression, leading to the transcriptional activation of all lipogenic genes (Youssef and McCullough, 2002). Simultaneously, hyperglycaemia activates ChREBP, which transcriptionally activates L-PK and all lipogenic genes. The synergistic actions of SREBP-1c and ChREBP co-ordinately activate the enzymatic machinery necessary for the conversion of excess glucose to fatty acids. A consequence of increased fatty acid synthesis is increased production of malonyl-CoA, which inhibits carnitine palmitoyl transferase 1(CPT), the protein responsible for fatty acid transport into the mitochondria. Thus, in the setting of insulin resistance, FFAs entering the liver from the periphery, as well as those derived from de novo lipogenesis, will be preferentially esterified to triglycerides.

The combination of hepatic steatosis, increased liver weights and increased hepatic index are some of the earliest features of Type 2 diabetes (Kotronen *et al*., 2008). Hepatic steatosis is often secondary to hepatic insulin resistance (Tolman *et al*., 2007). It is also known that in Type 2 Diabetes, the excess of substrate availability and the dysregulation of insulin signalling that occur mostly at the level of Insulin Receptor Substrate 2 (IRS 2) leads to hepatic accumulation of fat (Perry *et al*., 2014). The fat accumulation leads to steatosis, an increased liver weight and an increased liver weight: body weight ratio in Diabetes (Lucchesi *et al*., 2015).The aforementioned events ultimately result in hepatic accumulation of fat i.e. hepatic steatosis with the consequent increase in hepatic weight and index. One can therefore argue that compounds in the extract cause a reduction in hepatic triglyceride content and consequently prevent hepatic steatosis by inhibiting one or more of these biochemical pathways.

Insulin resistance plays an important role in development of diabetic dyslipidaemia by increasing efflux of free fatty acids from adipose, impairs insulin mediated skeletal muscle uptake of free fatty acids and increases fatty acid flux to the liver. The freeze-

dried extracts of *Rotheca myricoides* (Hochst.) Steane & Mabb possessed significant anti-dyslipidemic effects i.e. decreased total plasma cholesterol, LDL-cholesterol, serum triglyceride and increased HDL-cholesterol. The results obtained in this study are similar to those in published literature. Indeed, two plant species from Rotheca genus, *Rotheca capitatum* (A. A. Adeneye *et al*., 2008) and *Rotheca phlomidis* (Mohan and Mishra, 2010) were reported to possess significant anti-dyslipidemic effects.

The antihyperlipidemic effects of *Rotheca capitatum* and *Rotheca phlomidis* were directly attributed to the improvement in insulin signalling, activation of PPAR α receptor which activates the gene for liver acyl-CoA oxidase increasing fatty acid oxidation in the liver, this action lowers hepatic lipids and in effect, serum lipid levels, controlling and preventing the hyperlipidaemia seen in diabetes (Chao *et al*., 2011) and the antagonist activity at the bile acid receptor also called Farnesoid X Receptor (FXR) (Weng *et al*., 2014). The bile acid receptor inhibits cholesterol 7-alpha-monooxygenase or cytochrome P450 7A1 (CYP7A1) enzyme which catalyses the conversion of cholesterol to bile acid. This decreases blood cholesterol and improves the lipid profile (Bent, 2008). *Rotheca myricoides* may act in a similar manner.

The freeze-dried extracts of *Rotheca myricoides* (Hochst.) Steane & Mabb had significant serum uric acid lowering effects. It is known that elevated serum triglyceride is associated with hyperuricemia which results from the increased activity of the pentose phosphate cycle leading to increased NADPH requirement for new fatty acid synthesis. The increased NADPH requirements production leads to the enhancement of uric acid production (Chen *et al*., 2007). The hyperuricemia seen in high fat- high fructose group may also result from endothelial damage due to oxidative stress induced by hyperglycaemia, which subsequently leads to nephropathy hence decreased elimination of uric acid (Khosla *et al*., 2005). The reduction in serum uric acid exhibited by *Rotheca myricoides* (Hochst.) Steane & Mabb indirectly prevents insulin resistance by preventing the vicious cycle that may result from cellular oxidative stress and serine phosphorylation of IRS-2 from occurring.

The freeze-dried extracts of *Rotheca myricoides* (Hochst.) Steane & Mabb caused significant reductions in adipose tissue weights i.e. retroperitoneal fat, mesenteric fat

and pericardial fat. This correlates with published literature showing that the lipids in adipose tissue are mostly derived from circulating triglyceride and that reduction in serum triglyceride also leads to decreased adipose tissue mass (Yoon *et al*., 2003). A common characteristic of type 2 diabetes is high circulating levels of lipids, partly accounted for by impaired insulin-mediated suppression of lipolysis in adipose tissue. These free fatty acids deactivate the AMPK (Adenosine Monophosphate-activate Protein Kinase) pathway which is responsible for lipid metabolism in adipocytes (Woods *et al*., 2000). The AMPK pathway also appears to control whole-body adiposity hence the increase in weights of retroperitoneal fat, mesenteric fat and pericardial fat seen in type 2 diabetes (Long and Zierath, 2006).

Elevated circulating free fatty acids released by adipocytes are also associated with ectopic fat accumulation (Long and Zierath, 2006). One can therefore argue that freeze-dried extracts of *Rotheca myricoides* (Hochst.) Steane & Mabb cause a reduction in retroperitoneal fat, mesenteric fat and pericardial fat weights by activating the AMPK pathway and preventing the accumulation of ectopic fat. The freeze-dried extracts of *Rotheca myricoides* (Hochst.) Steane & Mabb possessed significant lowering effects on branched-chain amino acid serum levels. It has been well established that in both human and animal models of type 2 diabetes there is downregulation of adipose tissue branched-chain amino acid metabolizing enzyme (Sears *et al*., 2009). This is specifically caused by downregulation of the branched-chain ketoacid dehydrogenase complex (BCKDHC), that catalyses the oxidative decarboxylation of the α-ketoacids to their acyl-CoA esters (Herman *et al*., 2010). The branched-chain amino acid lowering effects of freeze-dried extracts of *Rotheca myricoides* (Hochst.) Steane & Mabb may have been attributed to increased expression of the branched-chain ketoacid dehydrogenase complex (BCKDHC), hence upregulation of adipose tissue branched-chain amino metabolizing enzyme (Sears *et al*., 2009).

5.2 RECOMMENDATIONS.

To further elucidate other mechanisms of action of freeze-dried extracts of *Rotheca myricoides* (Hochst.) Steane & Mabb, we recommend future studies to investigate its effects on:

1. Sodium-glucose cotransporter (SGLT 2) which is upregulated in type 2 diabetes mellitus.
2. Inflammatory markers such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tissue necrosis factor-alpha (TNF-α) which are associated with chronic inflammation observed in type 2 diabetes mellitus.
3. Levels of adipocytokines e.g. resistin, adiponectin and leptin which are often deranged in type 2 diabetes mellitus.

5.3 CONCLUSION

The freeze-dried extracts of *Rotheca myricoides* (Hochst.) Steane & Mabb possessed significant overall antidiabetogenesis effects by having significant beneficial effects on plasma glucose, insulin sensitivity, hepatic triglyceride, hepatic index, and lipid profile, serum uric acid, body weight, branched chain amino acids, adipose tissue weight and body weight. These results appear to validate its traditional uses in the management of diabetes mellitus and indicate that the main mechanism of action of these antidiabetic effects is via the increased expression of GLUT-4 in the insulin-dependent tissues. Future studies will focus on trying to isolate the chemical moiety(ies) responsible for mediating these beneficial pharmacological effects and determine the safety and efficacy in humans.

6.0 REFERENCES

Adeneye, A., Ajagbonna, O., Ayodele, O.W., 2008. Hypoglycemic and antidiabetic activities on the stem bark aqueous and ethanol extracts of Musanga cecropioides in normal and alloxan-induced diabetic rats. Fitoterapia 78, 502–5. https://doi.org/10.1016/j.fitote.2007.05.001

Adeneye, A.A., Adeleke, T.I., Adeneye, A.K., 2008a. Hypoglycemic and hypolipidemic effects of the aqueous fresh leaves extract of Clerodendrum capitatum in Wistar rats. J. Ethnopharmacol. 116, 7–10. https://doi.org/10.1016/j.jep.2007.10.029

Adeneye, A.A., Olagunju, J.A., Benebo, A.S., Elias, S.O., Adisa, A.O., Idowu, B.O., Oyedeji, M.O., Isioye, E.O., Braimoh, O.B., Oladejo, O.O., Alana, E.O., 2008b. Nephroprotective effects of the aqueous root extract of *Harungana madagascariensis* (L.) In acute and repeated dose acetaminophen renal injured rats. Int. J. Appl. Res. Nat. Prod. 1, 6–14.

Altan, V.M., 2003. The pharmacology of diabetic complications. Curr. Med. Chem. 10, 1317–1327. https://doi.org/10.2174/0929867033457287

Antunes, L.C., Elkfury, J.L., Jornada, M.N., Foletto, K.C., Bertoluci, M.C., 2016. Validation of HOMA-IR in a model of insulin-resistance induced by a high-fat diet in Wistar rats. Arch. Endocrinol. Metab. 60, 138–142. https://doi.org/10.1590/2359-3997000000169

Barrett, C., 2002. The glucose tolerance test: a pitfall in the diagnosis of diabetes mellitus. Adv. Intern. Med. 20, 297–323.

Bartoli, E., Fra, G.P., Carnevale Schianca, G.P., 2011. The oral glucose tolerance test (OGTT) revisited. Eur. J. Intern. Med. 22, 8–12. https://doi.org/10.1016/j.ejim.2010.07.008

Bashwira, S., Hootele, C., 1988. Myricoidine and dihydromyricoidine, two new macrocyclic spermidine alkaloids from clerodendrum myricoides. Tetrahedron 44, 4521–4526. https://doi.org/10.1016/S0040-4020(01)86153-6

Bent, S., 2008. Herbal Medicine in the United States: Review of Efficacy, Safety, and Regulation. J. Gen. Intern. Med. 23, 854–859. https://doi.org/10.1007/s11606-008-0632-y

Birech, Z., Mwangi, P.W., Bukachi, F., Mandela, K.M., 2017. Application of Raman spectroscopy in type 2 diabetes screening in blood using leucine and isoleucine amino-acids as biomarkers and in comparative anti-diabetic drugs efficacy studies. PloS One 12, e0185130. https://doi.org/10.1371/journal.pone.0185130

Butler, W.M., Maling, H.M., Horning, M.G., Brodie, B.B., 1961. The direct determination of liver triglycerides. J. Lipid Res. 2, 95–96.

Castro, M.C., Massa, M.L., Arbeláez, L.G., Schinella, G., Gagliardino, J.J., Francini, F., 2015. Fructose-induced inflammation, insulin resistance and oxidative stress: A liver pathological triad effectively disrupted by lipoic acid. Life Sci. 137, 1–6. https://doi.org/10.1016/j.lfs.2015.07.010

Chao, C.-Y., Yin, M.-C., Huang, C., 2011. Wild bitter gourd extract up-regulates mRNA expression of PPARα, PPARγ and their target genes in C57BL/6J mice. J. Ethnopharmacol. 135, 156–161. https://doi.org/10.1016/j.jep.2011.03.001

Chen, L., Magliano, D.J., Zimmet, P.Z., 2012. The worldwide epidemiology of type 2 diabetes mellitus—present and future perspectives. Nat. Rev. Endocrinol. 8, 228–236. https://doi.org/10.1038/nrendo.2011.183

Chen, L., Zhu, W., Chen, Z., Dai, H., Ren, J., Chen, J., Chen, L., Fang, L., 2007. Relationship between hyperuricemia and metabolic syndrome. J. Zhejiang Univ. Sci. B 8, 593–598. https://doi.org/10.1631/jzus.2007.B0593

Clark, J.D., Gebhart, G.F., Gonder, J.C., Keeling, M.E., Kohn, D.F., 1997. The 1996 Guide for the Care and Use of Laboratory Animals. ILAR J. 38, 41–48. https://doi.org/10.1093/ilar.38.1.41

Cnop, M., Welsh, N., Jonas, J.-C., Jörns, A., Lenzen, S., Eizirik, D.L., 2005. Mechanisms of Pancreatic β-Cell Death in Type 1 and Type 2 Diabetes: Many Differences, Few Similarities. Diabetes 54, S97–S107. https://doi.org/10.2337/diabetes.54.suppl_2.S97

Dabelea, D., Mayer-Davis, E.J., Saydah, S., Imperatore, G., Linder, B., Divers, J., Bell, R., Badaru, A., Talton, J.W., Crume, T., Liese, A.D., Merchant, A.T., Lawrence, J.M., Reynolds, K., Dolan, L., Liu, L.L., Hamman, R.F., 2014. Prevalence of Type 1 and Type 2 Diabetes Among Children and Adolescents From 2001 to 2009. JAMA 311, 1778–1786. https://doi.org/10.1001/jama.2014.3201

Das, S., Bhattacharya, S., Prasanna, A., Kumar, R.B.S., Pramanik, G., Haldar, P.K., 2011. Preclinical evaluation of antihyperglycemic activity of <Emphasis Type="Italic">Clerodendron infortunatum</Emphasis> leaf against streptozotocin-induced diabetic rats. Diabetes Ther. 2, 92–100. https://doi.org/10.1007/s13300-010-0019-z

DeFronzo, R.A., 2004. Pathogenesis of type 2 diabetes mellitus. Med. Clin. North Am. 88, 787–835, ix. https://doi.org/10.1016/j.mcna.2004.04.013

Dong, Y., Jing, T., Meng, Q., Liu, C., Hu, S., Ma, Y., Liu, Y., Lu, J., Cheng, Y., Wang, D., Teng, L., 2014. Studies on the antidiabetic activities of Cordyceps militaris extract in diet-streptozotocin-induced diabetic Sprague-Dawley rats. BioMed Res. Int. 2014, 160980. https://doi.org/10.1155/2014/160980

Esatu, H., Alemayehu, I., Haile, E., Tadesse, S., Mammo, F., Dekebo, A., Endale, M., 2015. Phenolic glycosides from roots of Clerodendrum myricoides 2.

Esubalew, S.T., Belete, A., Lulekal, E., Gabriel, T., Engidawor, E., Asres, K., 2017. Review of Ethnobotanical and Ethnopharmacological Evidences of some Ethiopian Medicinal Plants traditionally used for the Treatment of Cancer. Ethiop. J. Health Dev. EJHD 31.

Fan, M., Rhee, J., St-Pierre, J., Handschin, C., Puigserver, P., Lin, J., Jäeger, S., Erdjument-Bromage, H., Tempst, P., Spiegelman, B.M., 2004. Suppression of mitochondrial respiration through recruitment of p160 myb binding protein to PGC-1alpha: modulation by p38 MAPK. Genes Dev. 18, 278–289. https://doi.org/10.1101/gad.1152204

Ferrari, A.C., 2007. Raman spectroscopy of graphene and graphite: Disorder, electron–phonon coupling, doping and nonadiabatic effects. Solid State Commun., Exploring graphene 143, 47–57. https://doi.org/10.1016/j.ssc.2007.03.052

Flanagan, A.M., Brown, J.L., Santiago, C.A., Aad, P.Y., Spicer, L.J., Spicer, M.T., 2008. High-fat diets promote insulin resistance through cytokine gene expression in growing female rats. J. Nutr. Biochem. 19, 505–513. https://doi.org/10.1016/j.jnutbio.2007.06.005

Gan, Y., Yang, C., Tong, X., Sun, H., Cong, Y., Yin, X., Li, L., Cao, S., Dong, X., Gong, Y., Shi, O., Deng, J., Bi, H., Lu, Z., 2014. Shift work and diabetes mellitus: a meta-analysis of observational studies. Occup Env. Med oemed-2014-102150. https://doi.org/10.1136/oemed-2014-102150

Gerich, J.E., 1999. Is insulin resistance the principal cause of type 2 diabetes? Diabetes Obes. Metab. 1, 257–263. https://doi.org/10.1046/j.1463-1326.1999.00027.x

Ghasemi, A., Jeddi, S., 2017. Anti-obesity and anti-diabetic effects of nitrate and nitrite. Nitric Oxide 70, 9–24. https://doi.org/10.1016/j.niox.2017.08.003

Gheibi, S., Bakhtiarzadeh, F., Jeddi, S., Farrokhfall, K., Zardooz, H., Ghasemi, A., 2017a. Nitrite increases glucose-stimulated insulin secretion and islet insulin content in obese type 2 diabetic male rats. Nitric Oxide Biol. Chem. 64, 39–51. https://doi.org/10.1016/j.niox.2017.01.003

Gheibi, S., Kashfi, K., Ghasemi, A., 2017b. A practical guide for induction of type-2 diabetes in rat: Incorporating a high-fat diet and streptozotocin. Biomed. Pharmacother. Biomedecine Pharmacother. 95, 605–613. https://doi.org/10.1016/j.biopha.2017.08.098

Gleissner, C.A., Sanders, J.M., Nadler, J., Ley, K., 2008. Upregulation of Aldose Reductase During Foam Cell Formation as Possible Link Among Diabetes, Hyperlipidemia, and Atherosclerosis. Arterioscler. Thromb. Vasc. Biol. 28, 1137–1143. https://doi.org/10.1161/ATVBAHA.107.158295

Gong, Y., Wang, S., Liang, Z., Wang, Z., Zhang, X., Li, J., Song, J., Hu, X., Wang, K., He, Q., Bai, J., 2018. Label-Free Spectral Imaging Unveils Biochemical Mechanisms of Low-Level Laser Therapy on Spinal Cord Injury. Cell. Physiol. Biochem. 49, 1168–1183. https://doi.org/10.1159/000493295

Guevara, E., Torres-Galván, J.C., Ramírez-Elías, M.G., Luevano-Contreras, C., González, F.J., 2018. Use of Raman spectroscopy to screen diabetes mellitus with machine learning tools. Biomed. Opt. Express 9, 4998–5010. https://doi.org/10.1364/BOE.9.004998

Guillen, J., 2012. FELASA guidelines and recommendations. J. Am. Assoc. Lab. Anim. Sci. JAALAS 51, 311–321.

Guthrie, R.A., Guthrie, D.W., 2004. Pathophysiology of Diabetes Mellitus. Crit. Care Nurs. Q. 27, 113.

Herman, M.A., She, P., Peroni, O.D., Lynch, C.J., Kahn, B.B., 2010. Adipose tissue branched-chain amino acid (BCAA) metabolism modulates circulating BCAA levels. J. Biol. Chem. jbc.M109.075184. https://doi.org/10.1074/jbc.M109.075184

Hruz, P.W., Murata, H., Qiu, H., Mueckler, M., 2002. Indinavir induces acute and reversible peripheral insulin resistance in rats. Diabetes 51, 937–942.

Hu, F.B., Dam, R.M. van, Liu, S., 2001. Diet and risk of Type II diabetes: the role of types of fat and carbohydrate. Diabetologia 44, 805–817. https://doi.org/10.1007/s001250100547

Huffman, K.M., Shah, S.H., Stevens, R.D., Bain, J.R., Muehlbauer, M., Slentz, C.A., Tanner, C.J., Kuchibhatla, M., Houmard, J.A., Newgard, C.B., Kraus, W.E., 2009. Relationships between circulating metabolic intermediates and insulin action in overweight to obese, inactive men and women. Diabetes Care 32, 1678–1683. https://doi.org/10.2337/dc08-2075

Hui, H., Tang, G., Go, V., 2009a. Hypoglycemic herbs and their action mechanisms. Chin. Med. 4, 11. https://doi.org/10.1186/1749-8546-4-11

Hui, H., Tang, G., Go, V.L.W., 2009b. Hypoglycemic herbs and their action mechanisms. Chin. Med. 4, 11. https://doi.org/10.1186/1749-8546-4-11

IDF Diabetes Atlas Group, null, 2015. Update of mortality attributable to diabetes for the IDF Diabetes Atlas: Estimates for the year 2013. Diabetes Res. Clin. Pract. 109, 461–465. https://doi.org/10.1016/j.diabres.2015.05.037

Insel, R.A., Dunne, J.L., Atkinson, M.A., Chiang, J.L., Dabelea, D., Gottlieb, P.A., Greenbaum, C.J., Herold, K.C., Krischer, J.P., Lernmark, Å., Ratner, R.E., Rewers, M.J., Schatz, D.A., Skyler, J.S., Sosenko, J.M., Ziegler, A.-G., 2015. Staging Presymptomatic Type 1 Diabetes: A Scientific Statement of JDRF, the Endocrine Society, and the American Diabetes Association. Diabetes Care 38, 1964–1974. https://doi.org/10.2337/dc15-1419

Inzucchi, S.E., Bergenstal, R.M., Buse, J.B., Diamant, M., Ferrannini, E., Nauck, M., Peters, A.L., Tsapas, A., Wender, R., Matthews, D.R., 2015. Management of Hyperglycemia in Type 2 Diabetes, 2015: A Patient-Centered Approach: Update to a Position Statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care 38, 140–149. https://doi.org/10.2337/dc14-2441

Jadeja, R.N., Thounaojam, M.C., Ansarullah, A., Devkar, R.V., Ramachandran, A.V., 2009. A preliminary study on hypolipidemic effect of aqueous leaf extract of Clerodendron glandulosum.Coleb. Int. J. Green Pharm. IJGP 3. https://doi.org/10.22377/ijgp.v3i4.102

Jeruto, P., Mutai, C., Lukhoba, C., George, O., 2011. Phytochemical constituents of some medicinal plants used by the Nandis of South Nandi District, Kenya. J Anim Plant Sci 9, 1201–1210.

Jiménez-Agüero, R., Emparanza, J.I., Beguiristain, A., Bujanda, L., Alustiza, J.M., García, E., Hijona, E., Gallego, L., Sánchez-González, J., Perugorria, M.J., Asensio, J.I., Larburu, S., Garmendia, M., Larzabal, M., Portillo, M.P., Aguirre, L., Banales, J.M., 2014. Novel equation to determine the hepatic triglyceride concentration in humans by MRI: diagnosis and monitoring of NAFLD in obese patients before and after bariatric surgery. BMC Med. 12. https://doi.org/10.1186/s12916-014-0137-y

Joya, K.S., Sala, X., 2015. In situ Raman and surface-enhanced Raman spectroscopy on working electrodes: spectroelectrochemical characterization of water oxidation electrocatalysts. Phys. Chem. Chem. Phys. 17, 21094–21103. https://doi.org/10.1039/C4CP05053C

Kasper, J.S., Giovannucci, E., 2006. A Meta-analysis of Diabetes Mellitus and the Risk of Prostate Cancer. Cancer Epidemiol. Prev. Biomark. 15, 2056–2062. https://doi.org/10.1158/1055-9965.EPI-06-0410

Keter, L., Mutiso, P., 2012. Ethnobotanical studies of medicinal plants used by Traditional Health Practitioners in the management of diabetes in Lower Eastern Province, Kenya. J. Ethnopharmacol. 139, 74–80. https://doi.org/10.1016/j.jep.2011.10.014

Khosla, U.M., Zharikov, S., Finch, J.L., Nakagawa, T., Roncal, C., Mu, W., Krotova, K., Block, E.R., Prabhakar, S., Johnson, R.J., 2005. Hyperuricemia induces endothelial dysfunction. Kidney Int. 67, 1739–1742. https://doi.org/10.1111/j.1523-1755.2005.00273.x

King, A.J., 2012. The use of animal models in diabetes research. Br. J. Pharmacol. 166, 877–894. https://doi.org/10.1111/j.1476-5381.2012.01911.x

Kotronen, A., Juurinen, L., Tiikkainen, M., Vehkavaara, S., Yki–Järvinen, H., 2008. Increased Liver Fat, Impaired Insulin Clearance, and Hepatic and Adipose Tissue Insulin Resistance in Type 2 Diabetes. Gastroenterology 135, 122–130. https://doi.org/10.1053/j.gastro.2008.03.021

Lee, G., Goosens, K.A., 2015. Sampling Blood from the Lateral Tail Vein of the Rat. J. Vis. Exp. JoVE. https://doi.org/10.3791/52766

Long, Y.C., Zierath, J.R., 2006. AMP-activated protein kinase signaling in metabolic regulation. J. Clin. Invest. 116, 1776–1783. https://doi.org/10.1172/JCI29044

Lucchesi, A.N., Cassettari, L.L., Spadella, C.T., 2015. Alloxan-Induced Diabetes Causes Morphological and Ultrastructural Changes in Rat Liver that Resemble the Natural History of Chronic Fatty Liver Disease in Humans. J. Diabetes Res. 2015. https://doi.org/10.1155/2015/494578

Masih, I., McIlwaine, W., 2009. Non-alcoholic fatty liver disease in a patient with maturity onset diabetes in the young. BMJ Case Rep. 2009. https://doi.org/10.1136/bcr.07.2008.0436

Mayfield, J.A., 1998. Diagnosis and Classification of Diabetes Mellitus: New Criteria. Am. Fam. Physician 58, 1355.

Menke, A., Casagrande, S., Geiss, L., Cowie, C.C., 2015. Prevalence of and Trends in Diabetes Among Adults in the United States, 1988-2012. JAMA 314, 1021–1029. https://doi.org/10.1001/jama.2015.10029

Mittelman, S.D., Fu, Y.Y., Rebrin, K., Steil, G., Bergman, R.N., 1997. Indirect effect of insulin to suppress endogenous glucose production is dominant, even with hyperglucagonemia. J. Clin. Invest. 100, 3121–3130.

Mohan Maruga Raja, M., 2010. Comprehensive review of Clerodendrum phlomidis: a traditionally used bitter. J. Chin. Integr. Med. 8, 510–524. https://doi.org/10.3736/jcim20100602

Mohan, M.M.R., Mishra, S.H., 2010. Comprehensive review of Clerodendrum phlomidis: a traditionally used bitter. Zhong Xi Yi Jie He Xue Bao 8, 510–524.

Moshi, M.J., Otieno, D.F., Weisheit, A., 2012. Ethnomedicine of the Kagera Region, north western Tanzania. Part 3: plants used in traditional medicine in Kikuku village, Muleba District. J. Ethnobiol. Ethnomedicine 8, 14. https://doi.org/10.1186/1746-4269-8-14

Moszynski, P., 2006. WHO report highlights Africa's health challenges. BMJ 333, 1088. https://doi.org/10.1136/bmj.39041.697824.6C

Mukungu, N., Abuga, K., Okalebo, F., Ingwela, R., Mwangi, J., 2016. Medicinal plants used for management of malaria among the Luhya community of Kakamega East sub-County, Kenya. J. Ethnopharmacol. 194, 98–107. https://doi.org/10.1016/j.jep.2016.08.050

Newgard, C.B., 2012. Interplay between Lipids and Branched-Chain Amino Acids in Development of Insulin Resistance. Cell Metab. 15, 606–614. https://doi.org/10.1016/j.cmet.2012.01.024

Pan, D.A., Storlien, L.H., 1993. Dietary Lipid Profile Is a Determinant of Tissue Phospholipid Fatty Acid Composition and Rate of Weight Gain in Rats. J. Nutr. 123, 512–519. https://doi.org/10.1093/jn/123.3.512

Perreault, L., Newsom, S.A., Strauss, A., Kerege, A., Kahn, D.E., Harrison, K.A., Snell-Bergeon, J.K., Nemkov, T., D'Alessandro, A., Jackman, M.R., MacLean, P.S., Bergman, B.C., n.d. Intracellular localization of diacylglycerols and sphingolipids influences insulin sensitivity and mitochondrial function in human skeletal muscle. JCI Insight 3. https://doi.org/10.1172/jci.insight.96805

Perry, R.J., Camporez, J.-P.G., Kursawe, R., Titchenell, P.M., Zhang, D., Perry, C.J., Jurczak, M.J., Abudukadier, A., Han, M.S., Zhang, X.-M., Ruan, H.-B., Yang, X., Caprio, S., Kaech, S.M., Sul, H.S., Birnbaum, M.J., Davis, R.J., Cline, G.W., Petersen, K.F., Shulman, G.I., 2015. Hepatic acetyl CoA links adipose tissue inflammation to hepatic insulin resistance and type 2 diabetes. Cell 160, 745–758. https://doi.org/10.1016/j.cell.2015.01.012

Perry, R.J., Samuel, V.T., Petersen, K.F., Shulman, G.I., 2014. The role of hepatic lipids in hepatic insulin resistance and type 2 diabetes. Nature 510, 84. https://doi.org/10.1038/nature13478

Petro, A.E., Cotter, J., Cooper, D.A., Peters, J.C., Surwit, S.J., Surwit, R.S., 2004. Fat, carbohydrate, and calories in the development of diabetes and obesity in the C57BL/6J mouse. Metabolism. 53, 454–457.

Podell, B.K., Ackart, D.F., Richardson, M.A., DiLisio, J.E., Pulford, B., Basaraba, R.J., 2017. A model of type 2 diabetes in the guinea pig using sequential diet-induced glucose intolerance and streptozotocin treatment. Dis. Model. Mech. 10, 151–162. https://doi.org/10.1242/dmm.025593

Richard, S., Maciuk, A., Banzouzi, J., Champy, P., Figadere, B., Guissou, I.P., Nacoulma, O.G., 2011. Mutagenic effect, antioxidant and anticancer activities of six medicinal plants from Burkina Faso. https://doi.org/10.1080/14786419.2010.534737

Richards, M.R., Harp, J.D., Ory, D.S., Schaffer, J.E., 2006. Fatty acid transport protein 1 and long-chain acyl coenzyme A synthetase 1 interact in adipocytes. J. Lipid Res. 47, 665–672. https://doi.org/10.1194/jlr.M500514-JLR200

Rojas, L.B.A., Gomes, M.B., 2013. Metformin: an old but still the best treatment for type 2 diabetes. Diabetol. Metab. Syndr. 5, 6. https://doi.org/10.1186/1758-5996-5-6

Sears, D.D., Hsiao, G., Hsiao, A., Yu, J.G., Courtney, C.H., Ofrecio, J.M., Chapman, J., Subramaniam, S., 2009. Mechanisms of human insulin resistance and thiazolidinedione-mediated insulin sensitization. Proc. Natl. Acad. Sci. 106, 18745–18750. https://doi.org/10.1073/pnas.0903032106

Shaw, J.E., Sicree, R.A., Zimmet, P.Z., 2010. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res. Clin. Pract. 87, 4–14. https://doi.org/10.1016/j.diabres.2009.10.007

Shepherd, P.R., Kahn, B.B., 1999. Glucose transporters and insulin action--implications for insulin resistance and diabetes mellitus. N. Engl. J. Med. 341, 248–257. https://doi.org/10.1056/NEJM199907223410406

Singh, B., Saxena, A., 2010. Surrogate markers of insulin resistance: A review. World J. Diabetes 1, 36–47. https://doi.org/10.4239/wjd.v1.i2.36

Skyler, J.S., Bakris, G.L., Bonifacio, E., Darsow, T., Eckel, R.H., Groop, L., Groop, P.-H., Handelsman, Y., Insel, R.A., Mathieu, C., McElvaine, A.T., Palmer, J.P., Pugliese, A., Schatz, D.A., Sosenko, J.M., Wilding, J.P.H., Ratner, R.E., 2017. Differentiation of Diabetes by Pathophysiology, Natural History, and Prognosis. Diabetes 66, 241–255. https://doi.org/10.2337/db16-0806

Srinivasan, K., Ramarao, P., 2007a. Animal models in type 2 diabetes research: an overview. Indian J. Med. Res. 125, 451–472.

Srinivasan, K., Ramarao, P., 2007b. Animal models in type 2 diabetes research: an overview. Indian J. Med. Res. 125, 451–472.

Steane, D.A., Mabberley, D.J., 1998. Rotheca (Lamiaceae) Revived. Novon 8, 204–206. https://doi.org/10.2307/3391997

Strojek, K., 2003. Features of macrovascular complications in type 2 diabetic patients. Acta Diabetol. 40, s334–s337. https://doi.org/10.1007/s00592-003-0115-x

Szkudelski, T., 2001. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol. Res. 50, 537–546.

Titchenell, P.M., Lazar, M.A., Birnbaum, M.J., 2017. Unraveling the Regulation of Hepatic Metabolism by Insulin. Trends Endocrinol. Metab. TEM 28, 497–505. https://doi.org/10.1016/j.tem.2017.03.003

Tolman, K.G., Fonseca, V., Dalpiaz, A., Tan, M.H., 2007. Spectrum of Liver Disease in Type 2 Diabetes and Management of Patients With Diabetes and Liver Disease. Diabetes Care 30, 734–743. https://doi.org/10.2337/dc06-1539

Wachtel-Galor, S., Benzie, I.F.F., 2011. Herbal Medicine: An Introduction to Its History, Usage, Regulation, Current Trends, and Research Needs, in: Benzie, I.F.F., Wachtel-Galor, S. (Eds.), Herbal Medicine: Biomolecular and Clinical Aspects. CRC Press/Taylor & Francis, Boca Raton (FL).

Weng, Y., Yu, L., Cui, J., Zhu, Y.-R., Guo, C., Wei, G., Duan, J.-L., Yin, Y., Guan, Y., Wang, Y.-H., Yang, Z.-F., Xi, M.-M., Wen, A.-D., 2014. Antihyperglycemic, hypolipidemic and antioxidant activities of total saponins extracted from Aralia taibaiensis in experimental type 2 diabetic rats. J. Ethnopharmacol. 152, 553–560. https://doi.org/10.1016/j.jep.2014.02.001

Winzell, M.S., Ahrén, B., 2004. The High-Fat Diet–Fed Mouse: A Model for Studying Mechanisms and Treatment of Impaired Glucose Tolerance and Type 2 Diabetes. Diabetes 53, S215–S219. https://doi.org/10.2337/diabetes.53.suppl_3.S215

Woods, A., Azzout-Marniche, D., Foretz, M., Stein, S.C., Lemarchand, P., Ferré, P., Foufelle, F., Carling, D., 2000. Characterization of the Role of AMP-Activated Protein Kinase in the Regulation of Glucose-Activated Gene Expression Using Constitutively Active and Dominant Negative Forms of the Kinase. Mol. Cell. Biol. 20, 6704–6711. https://doi.org/10.1128/MCB.20.18.6704-6711.2000

Yeckel, C.W., Weiss, R., Dziura, J., Taksali, S.E., Dufour, S., Burgert, T.S., Tamborlane, W.V., Caprio, S., 2004. Validation of Insulin Sensitivity Indices from Oral Glucose Tolerance Test Parameters in Obese Children and Adolescents. J. Clin. Endocrinol. Metab. 89, 1096–1101. https://doi.org/10.1210/jc.2003-031503

Yoon, M., Jeong, S., Lee, H., Han, M., Kang, J.H., Kim, E.Y., Kim, M., Oh, G.T., 2003. Fenofibrate improves lipid metabolism and obesity in ovariectomized LDL receptor-null mice. Biochem. Biophys. Res. Commun. 302, 29–34. https://doi.org/10.1016/S0006-291X(03)00088-3

Youssef, W.I., McCullough, A.J., 2002. Steatohepatitis in obese individuals. Best Pract. Res. Clin. Gastroenterol. 16, 733–747. https://doi.org/10.1053/bega.2002.0334

APPENDICES

Appendix 1: Fasting blood glucose (mmol/L)

Week 0

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	4.6	4.2	4	3.8
Rat 2	4.1	4.6	4.7	4.6
Rat 3	4	4.6	4. 1	4.4
Rat 4	4.6	4.1	5.3	4.2
Rat 5	4.8	4.4	4.7	4
Rat 6	4.9	4.6	4.6	4.3
Rat 7	4.4	4.7	4.4	4.4
Rat 8	4.6	3.9	4	4.9
Rat 9	4	4.3	4	4.5
Rat 10	4.2	4.7	4.5	4.6

Week 1

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	4.3	4	4.4	4.2
Rat 2	4.1	4	4.1	4
Rat 3	4.2	4	4	4
Rat 4	4.3	4.2	4	4
Rat 5	4.3	4	4	4.1
Rat 6	4.1	4	4.2	4.4
Rat 7	4.5	4.4	4	4
Rat 8	4.4	4	4.1	4.1
Rat 9	4.1	4.4	4.6	4.2
Rat 10	4.2	3.8	4.1	4

Week 2

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	4.8	4.1	4.3	3.9
Rat 2	4.7	4.2	4.6	4
Rat 3	4.4	3.8	4.1	3.2
Rat 4	4.2	4	4.4	3.9

Rat 5	5	4.8	4.9	4.6
Rat 6	4.7	3.8	4	3.8
Rat 7	4.4	4.1	4.1	4. 4
Rat 8	4.9	3.8	4	3.6
Rat 9	4.5	4.3	4.2	4.8
Rat 10	4.8	4.5	5	4

Week 3

Rat number	**Negative control**	**Low dose test (50mg)**	**High dose test (100mg)**	**Positive control**
Rat 1	4.9	4.2	4.5	4
Rat 2	5.3	4.4	4.8	3.8
Rat 3	4.9	4.3	4.5	4.5
Rat 4	5.1	3.6	3.9	4
Rat 5	5.5	4.4	5. 2	4.4
Rat 6	4.8	4.1	4.3	4.3
Rat 7	5.3	4.6	4.1	4.7
Rat 8	5.1	3.9	4.9	4
Rat 9	4.8	3.8	4.5	3.4
Rat 10	4.9	4.4	4.3	4.1

Week 4

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	6.3	5	5	5
Rat 2	6.1	4.2	5.6	4.8
Rat 3	5.5	5.3	4.7	4.6
Rat 4	6.6	5	5.4	4.2
Rat 5	6.3	4.6	5.1	5.1
Rat 6	5.2	4.7	5.8	4.7
Rat 7	5.7	5.1	4.9	5.1
Rat 8	5.9	4.5	5.1	4.6
Rat 9	6.8	4	4.3	4.3
Rat 10	7	4.1	5.8	4.2

Week 5

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	6.9	4.1	5.7	4.2
Rat 2	5.8	5	5.4	4.4
Rat 3	6.6	4.4	5.3	4.8
Rat 4	6.1	4.7	5.8	4

Rat 5	6.8	4.6	4.9	4.4
Rat 6	6.6	4.5	4.9	4.5
Rat 7	6.2	4.9	4.4	4.1
Rat 8	6.9	5.4	4.8	3.9
Rat 9	6.5	4.1	5.9	4.6
Rat 10	6.6	4.5	5.4	4.4

Week 6

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	7.2	3.6	5.3	4.4
Rat 2	7.1	4.6	5	4.4
Rat 3	6.9	4.3	4.3	3.8
Rat 4	7	4.1	4	3.7
Rat 5	7.1	3.7	3.6	4.7
Rat 6	7.2	3.9	4	4.2
Rat 7	7.4	4.1	4.6	4
Rat 8	6.6	4	4.4	3.4
Rat 9	7.5	3.7	4.8	4
Rat 10	7.9	4.2	4.3	4.5

Week 7

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	7.1	3.8	3.7	3.3
Rat 2	6.8	3.6	4.3	4
Rat 3	8.3	4	3.8	4.1
Rat 4	6.9	3.8	4	3.2
Rat 5	8.4	4.1	3.4	3.7
Rat 6	8.4	3.2	4.1	4.2
Rat 7	7.2	3.7	3.4	3.6
Rat 8	7.7	4.1	4.1	4.2
Rat 9	8.4	3.6	3.8	3.9
Rat 10	8.8	3.9	4.6	4.4

Week 8

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	7.3	3.6	3.1	3.4
Rat 2	7.4	4.1	4.1	3.6
Rat 3	8.2	3.6	3.5	4
Rat 4	8.1	3.4	4.1	3.3

Rat 5	8.7	3.6	3.8	3.3
Rat 6	8.3	3.1	3.9	3.8
Rat 7	8	3.5	3.7	3.6
Rat 8	7.8	3.8	3.5	4
Rat 9	8.1	3.3	3.5	3.7
Rat 10	8.7	3.4	4.1	4.1

Appendix 2: Oral glucose tolerance test

Week 4: area under the curve for OGTT (mmol/L X mins)

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	1030.5	841.5	894	868.5
Rat 2	1134	888	838.5	759
Rat 3	898.5	790.5	928.5	879
Rat 4	1050	777	972	819
Rat 5	1027.5	913.5	813	853.5
Rat 6	841.5	790.5	939	904.5
Rat 7	1029	754.5	768	781.5
Rat 8	1120.5	910.5	958.5	801
Rat 9	1090.5	799.5	774	781.5
Rat 10	1177.5	811.5	921	823.5

Area under the curve for OGTT (mmol/L X mins)

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	1614	736.5	777	778.5
Rat 2	1243.5	789	919.5	814.5
Rat 3	1395	714	846	828
Rat 4	1705.5	660	844.5	807
Rat 5	1774.5	901.5	822	832.5
Rat 6	1659	745.5	850.5	756
Rat 7	1608	780	879	777
Rat 8	1773	823.5	835.5	745.5
Rat 9	1654.5	703.5	921	795
Rat 10	1828.5	651	918	883.5

Appendix 3: Fasting insulin levels (mU/L)

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	1.2	0.37	1.4	0.3
Rat 2	0.9	1.3	0.32	0.5
Rat 3	2.4	0.9	1.2	1.3
Rat 4	2.8	0.26	1.5	0.98
Rat 5	1.8	1.4	0.7	1.0
Rat 6	2.3	0.3	0.4	1.13
Rat 7	1.1	0.6	0.32	0.2
Rat 8	1.87	0.8	0.6	0.28
Rat 9	1.9	0.6	1.6	0.33
Rat 10	2.1	0.4	0.3	0.9

Appendix 4: Homeostatic model assessment of insulin resistance (HOMA-IR) score

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	3.3	1.2	0.8	0.9
Rat 2	2.7	0.4	0.9	0.5
Rat 3	2.9	0.8	0.8	0.6
Rat 4	3.3	0.5	1.1	0.7
Rat 5	2.4	0.6	0.9	1.1
Rat 6	2.9	0.4	0.8	0.7
Rat 7	3.2	1	0.5	0.3
Rat 8	2.8	0.8	1	0.5
Rat 9	3.5	1.1	0.8	0.4
Rat 10	3	0.8	1.3	0.5

Appendix 5: Hepatic triglycerides (mg/g)

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	5.2	2.7	2.5	1. 6
Rat 2	4.9	2.4	3.1	2.1
Rat 3	4.7	2.1	2.7	2.2
Rat 4	4.8	2	2.3	2
Rat 5	5.8	1.8	2.2	1.9
Rat 6	6.1	2.5	2.1	1. 6
Rat 7	4.7	1.9	2	2.1
Rat 8	4.8	1.7	2.2	2
Rat 9	5.1	2.2	1.9	1.9
Rat 10	4.8	2.1	2	2

Appendix 6: Serum lipid profile

Total cholesterol (mmol/L)

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	5.5	2	2.1	2.1
Rat 2	5.5	1.6	2	1.6
Rat 3	6.1	2.5	1.5	1.8
Rat 4	5.4	1.2	1.1	1.4
Rat 5	5.8	1.1	1.8	1.7
Rat 6	5.2	1.4	1.6	1.8
Rat 7	5.2	2.1	1.9	1.6
Rat 8	5.7	1.3	1.8	1.4
Rat 9	6.1	1.4	1.1	2.1
Rat 10	5.9	1.6	1.9	1.1

HDL cholesterol (mmol/L)

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	0.9	1.96	2.1	1.85
Rat 2	0.54	1.6	1.15	2.1
Rat 3	0.32	2.4	1.65	2.61
Rat 4	0.67	1.45	1.79	1.56
Rat 5	1.08	1.34	1.56	1.96
Rat 6	0.87	1.08	1.87	1.04
Rat 7	0.9	1.76	1.58	1.54
Rat 8	0.54	1.95	1.87	1.08
Rat 9	1.13	1.56	1.65	1.56
Rat 10	0.89	1.45	1.77	1.5 5

LDL cholesterol (mmol/L)

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	3.36	0.34	0.31	0.41
Rat 2	2.5	0.32	0.29	0.26
Rat 3	3.1	0.27	0.22	0.36
Rat 4	2.99	0.31	0.34	0.4
Rat 5	4.11	0.41	0.41	0.31
Rat 6	3.98	0.34	0.3	0.28
Rat 7	3.9	0.33	0.41	0.32
Rat 8	4.52	0.32	0.39	0.34
Rat 9	3.45	0.26	0.34	0.33
Rat 10	3.31	0.36	0.41	0.32

Serum triglycerides (mmol/L)

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	3.12	0.8	1.1	0.43
Rat 2	3.15	0.3	0.93	0.84
Rat 3	4.3	0.5	0.84	1.16
Rat 4	3.1	0.7	1.12	0.94
Rat 5	3	0.83	0.29	0.26
Rat 6	3.9	0.91	0.32	0.62
Rat 7	3.82	0.6	0.51	0.78
Rat 8	4.8	0.56	0.42	0.31
Rat 9	4.67	0.32	0.67	0.89
Rat 10	4.4	0.45	0.54	0.64

Appendix 7: Area under the curve for mechanism of action (OGTT) (mmol/L X mins)

Rat number	Negative control	Positive control	Test group I	Test group II
Rat 1	667.8	462	718.5	750
Rat 2	684	408	762	694.5
Rat 3	745.3	420	688.5	813
Rat 4	734.8	369	654	786.2
Rat 5	645	478.5	730.5	783
Rat 6	748.4	426	688.5	745.7

Appendix 8: Serum uric acid (mg/dL)

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	2.6	0.5	0.4	0.2
Rat 2	2.1	0.3	0.7	0.6
Rat 3	3.5	0.2	0.5	0.7
Rat 4	2.6	0.3	0.31	0.9
Rat 5	1.9	0.12	0.4	0.2
Rat 6	2.5	0.2	0.6	0.4
Rat 7	2	0.4	0.8	0.7

Rat 8	3.3	0.6	0.4	0.2
Rat 9	3.8	0.3	0.3	0.5
Rat 10	2.7	0.4	0.5	0.4

Appendix 9: Hepatic index

Liver weights (g)

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	8.21	3.12	3.34	3.23
Rat 2	8.56	4.01	3.31	3.21
Rat 3	7.91	2.98	3.09	3.02
Rat 4	9.01	3.85	3.54	3.46
Rat 5	7.39	3.99	3.93	3.31
Rat 6	8.98	3.82	3.64	3.67
Rat 7	9.11	3.19	3.72	3.12
Rat 8	8.02	2.96	4.06	3.11
Rat 9	8.8	3.87	3.72	3.88
Rat 10	9.54	3.04	4.05	3.06

Liver weight: body weight ratio (%)

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	8.21	3.12	3.34	3.23
Rat 2	8.56	4.01	3.31	3.21
Rat 3	7.91	2.98	3.09	3.02
Rat 4	9.01	3.85	3.54	3.46
Rat 5	7.39	3.99	3.93	3.31
Rat 6	8.98	3.82	3.64	3.67
Rat 7	9.11	3.19	3.72	3.12
Rat 8	8.02	2.96	4.06	3.11
Rat 9	8.8	3.87	3.72	3.88
Rat 10	9.54	3.04	4.05	3.06

Appendix 10: Branched chain amino acids (arbitrary units)

Raman intensity of leucine

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	0.8	0.32	0.3	0.321
Rat 2	0.93	0.341	0.431	0.324
Rat 3	0.82	0.423	0.321	0.243
Rat 4	0.805	0.333	0.232	0.321
Rat 5	0.812	0.231	0.321	0.436
Rat 6	0.814	0.431	0.21	0.256

Raman intensity of isoleucine

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	0.762	0.41	0.321	0.311
Rat 2	0.653	0.221	0.458	0.376
Rat 3	0.845	0.321	0.317	0.211
Rat 4	0.961	0.243	0.289	0.25
Rat 5	0.761	0.431	0.354	0.372
Rat 6	0.844	0.112	0.201	0.249

Raman intensity of valine

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	0.8	0.32	0.3	0.321
Rat 2	0.93	0.341	0.431	0.324
Rat 3	0.82	0.423	0.321	0.243
Rat 4	0.805	0.333	0.232	0.321
Rat 5	0.812	0.231	0.321	0.436
Rat 6	0.814	0.431	0.21	0.256

Raman intensity of creatine monohydrate

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	0.8	0.32	0.3	0.321
Rat 2	0.93	0.341	0.431	0.324
Rat 3	0.82	0.423	0.321	0.243
Rat 4	0.805	0.333	0.232	0.321
Rat 5	0.812	0.231	0.321	0.436
Rat 6	0.814	0.431	0.21	0.256

Appendix 11: Adipose tissue weights (g)

Retroperitoneal fat

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	6.7	2.7	4.5	6.7
Rat 2	7.7	4.6	4.1	8.4
Rat 3	7.6	2.8	2.6	7.9
Rat 4	8.9	2.6	1.9	10
Rat 5	6.9	5.4	3.7	11.7
Rat 6	8.8	3.2	2.8	9.9
Rat 7	9.8	2.8	5.3	8.7
Rat 8	7.5	3.7	3.7	7.7
Rat 9	7.9	4.8	4.9	9.9
Rat 10	8.7	4.3	4.8	11.7

Pericardial fat

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	2.1	0.9	0.5	2.2
Rat 2	1.9	0.6	0.8	1.9
Rat 3	3.4	1.1	0.8	3.8
Rat 4	3	0.6	1.3	2.6
Rat 5	2.6	0.4	0.9	2.6
Rat 6	3.4	1.5	0.6	2.1
Rat 7	3.5	2	0.5	3.4
Rat 8	2.4	0.9	0.6	3.3
Rat 9	2.5	0.8	1.4	2.7
Rat 10	3.1	1.1	0.8	2.2

Mesenteric fat

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	10	6.7	6.1	12.9
Rat 2	11.2	5.8	6.7	11.7
Rat 3	14.7	7.7	7.1	15.7
Rat 4	12.4	6.9	6.7	8.9
Rat 5	9.8	5.6	6.4	16.3
Rat 6	12.6	6.8	6.5	15.4
Rat 7	13.5	7.8	7.5	13.3
Rat 8	12.5	5.8	6.7	13.7
Rat 9	11.6	6.4	5.4	11.6
Rat 10	13.6	6.7	5.7	14.1

Appendix 12: Weekly body weight (g)

Week 0

Rat number	Negative control	Low dos test (50mg)	High dose test (100mg)	Positive control
Rat 1	100.1	91.6	122.7	94.1
Rat 2	98	113	120	131.1
Rat 3	70.7	97.7	112.7	140
Rat 4	73.9	85.1	115.9	74
Rat 5	90.6	87.2	99.7	61.9
Rat 6	100	114.2	82.4	109.7
Rat 7	133.7	113.8	82.4	132.9
Rat 8	109.7	106.7	81.7	83.8
Rat 9	95.6	85.5	108.5	125.5
Rat 10	131.5	110.2	115	110.5

Week 1

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	126.5	110.5	143.5	125.5
Rat 2	101.8	130.9	150	126.2
Rat 3	113.6	107.6	149.1	100.4
Rat 4	107.1	112.1	154.9	103.1
Rat 5	130.3	107.4	123.6	100.2
Rat 6	78.2	136.1	100.8	138.2
Rat 7	168.5	140.2	84.7	161.7
Rat 8	108.4	124.9	100.8	102.7
Rat 9	124.1	107.7	113.8	159.7
Rat 10	150.6	136.9	152.7	137.2

Week 2

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	158.2	111.9	171.4	146.9
Rat 2	140.1	164.7	177.2	172
Rat 3	159.6	132.7	182.4	134.6
Rat 4	128.5	101.7	200.5	127.8

Rat 5	165.1	145.5	151	133.8
Rat 6	129.1	149.4	114.8	164.6
Rat 7	195.5	170.7	93.9	190.7
Rat 8	129.9	145	117.2	127.3
Rat 9	139.4	130.9	106.9	192.6
Rat 10	158.9	160.8	181.1	167.5

Week 3

Rat number	**Negative control**	**Low dose test (50mg)**	**High dose test (100mg)**	**Positive control**
Rat 1	195.5	131.1	191.2	148.9
Rat 2	163.1	174.8	186.3	187.7
Rat 3	179.1	126.1	188.5	151.1
Rat 4	156.7	105.2	227.5	142.1
Rat 5	192.7	164.4	166.9	159.8
Rat 6	135.7	149.5	120.3	178.6
Rat 7	199.6	149.4	111.2	206.6
Rat 8	152.5	178	123.7	139
Rat 9	146.8	142	109.1	110.5
Rat 10	165.7	155.1	196.9	180.8

Week 4

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	215	161.5	203.5	145.2
Rat 2	190.2	196.1	195.3	214.4
Rat 3	192.1	118.8	200.5	180.1
Rat 4	178	150.8	248.3	162.9
Rat 5	219	194.4	165.9	178.2
Rat 6	143.1	157.6	128	188.7
Rat 7	210.9	185.3	117.6	224.9
Rat 8	164.1	153.5	133	153.9
Rat 9	145.1	149.8	120.6	222.4
Rat 10	167.5	164	201.5	187.5

Week 5

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	253.9	195.7	219.7	256.1
Rat 2	234.9	231.5	229.4	242.2
Rat 3	208.5	236.1	241.1	265.3
Rat 4	240.8	236.1	251.8	195

Rat 5	262.8	145.1	187.5	251.3
Rat 6	247.1	165.4	210.4	245.8
Rat 7	242.9	186.7	200.2	245.5
Rat 8	234.5	169.2	151.3	213.6
Rat 9	224.3	162.9	204.6	243.7
Rat 10	216.5	177.2	217.4	258.1

Week 6

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	281.3	210.7	245.6	300.7
Rat 2	266.1	256.7	262.4	295.6
Rat 3	250.8	278	256.3	279.6
Rat 4	284.8	254.8	272.1	231
Rat 5	300.5	200.6	256.1	306.1
Rat 6	296.4	198.8	267.8	289.2
Rat 7	304.3	234.7	255.8	317.4
Rat 8	278.8	221.7	234.7	265.6
Rat 9	267.1	223.8	248.6	243.7
Rat 10	245.6	234.5	287.8	302.8

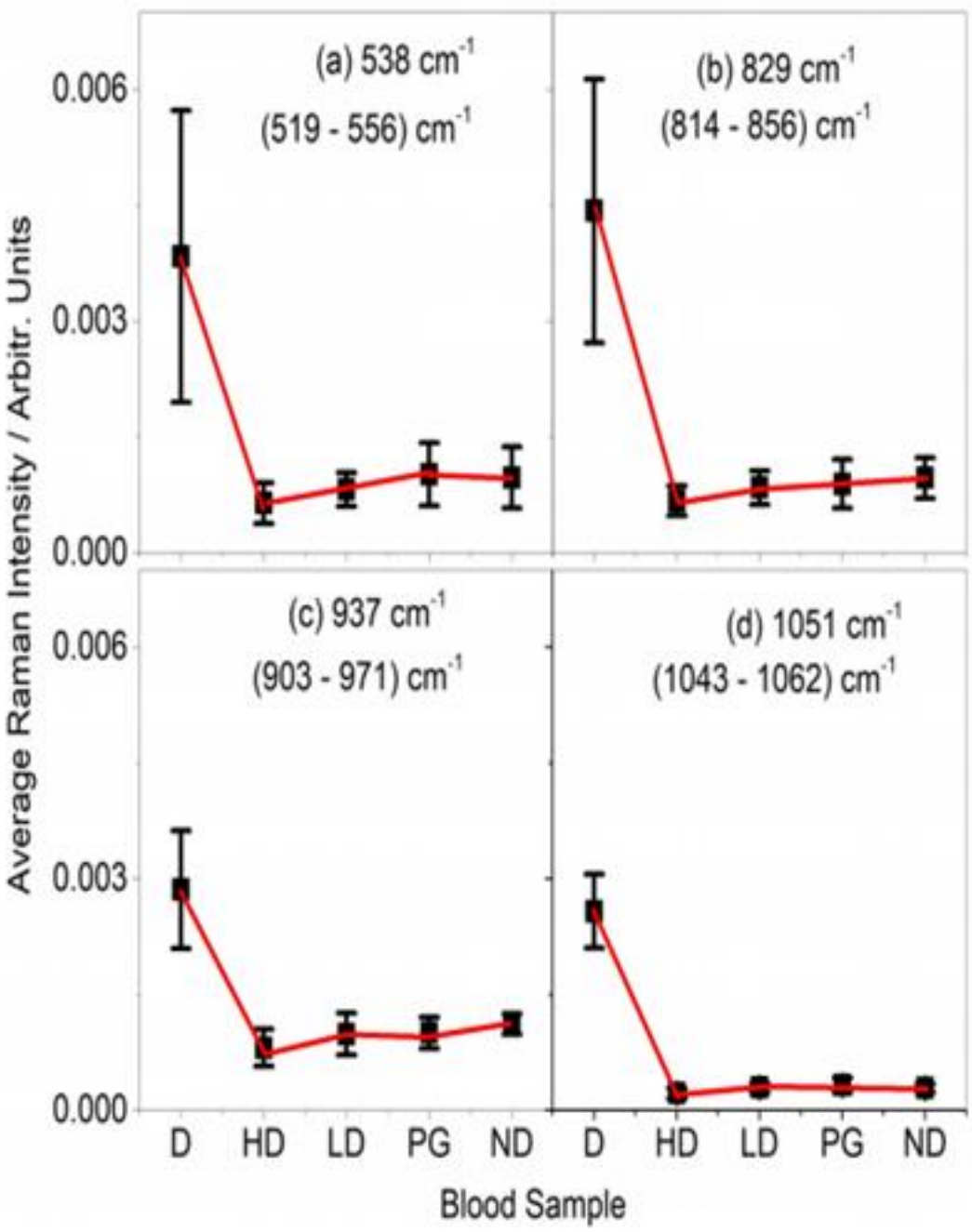
Week 7

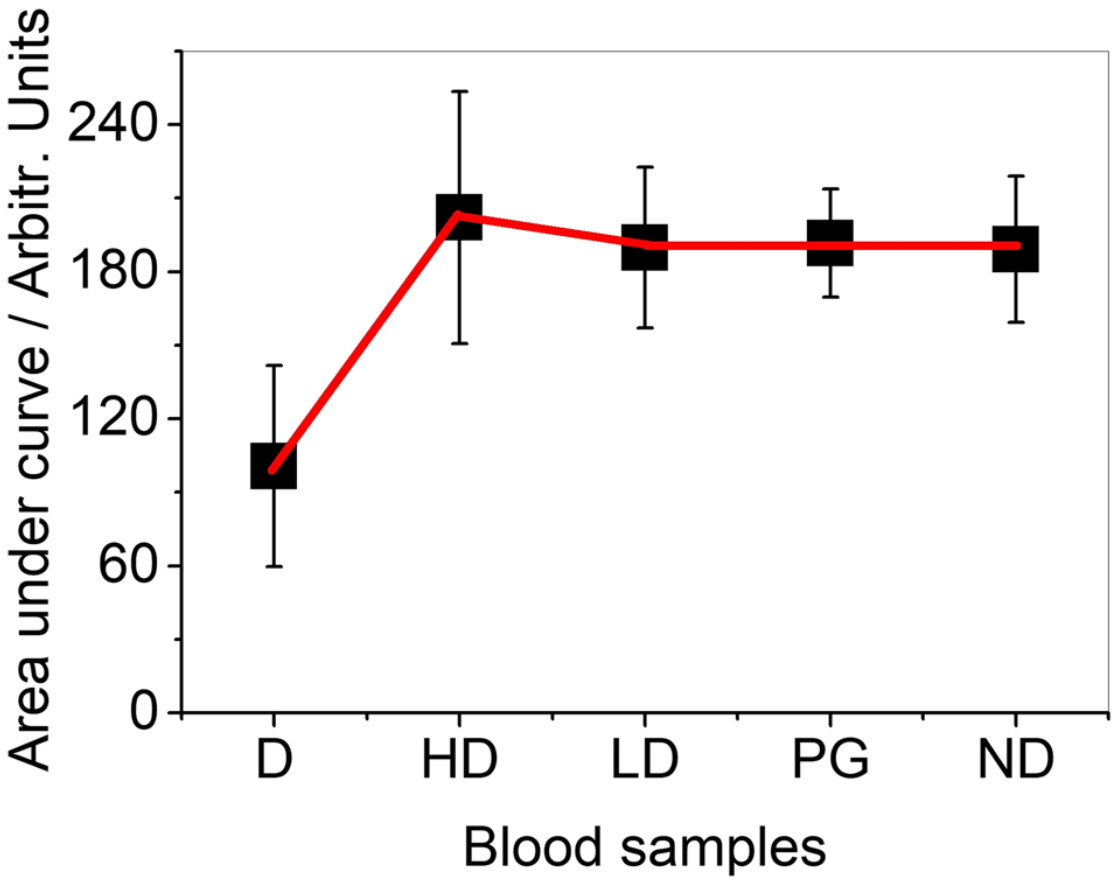
Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	319	241.2	298.4	323.8
Rat 2	299.1	287.5	311.5	345.9
Rat 3	315.4	303.4	301.7	343.6
Rat 4	345.8	297.7	289.4	298.2
Rat 5	337.1	248	303	341.7
Rat 6	314.9	219.6	289.3	310.7
Rat 7	341.1	308.8	286.4	351.2
Rat 8	300.6	264.7	262.5	300.4
Rat 9	327.5	300.2	276.7	307.8
Rat 10	311.6	286.8	300.8	344.6

Week 8

Rat number	Negative control	Low dose test (50mg)	High dose test (100mg)	Positive control
Rat 1	341.2	261.7	312.7	350.3
Rat 2	320.6	307.3	309.3	359.2
Rat 3	346.1	329	315.1	367.4
Rat 4	345.3	314.5	317.8	356.7
Rat 5	357.1	250.1	316.8	348.8
Rat 6	352.4	228.6	307.1	332.3
Rat 7	357.8	317.2	312.5	364.5
Rat 8	326.3	276.8	306	341.8
Rat 9	348.8	318.3	324.9	327.1
Rat 10	336.7	351.7	313.8	363

Appendix 13: Average Raman intensity of amino acid in experimental groups




Area under curve / Arbitr. Units
240
180
120
60
0
D
HD
LD
PG
ND
Blood samples