

ANTI-DIABETIC PROPERTIES OF GUH

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LIST OF ABBREVIATIONS

ADA – American Diabetes Association

ATP – Adenosine tri-phosphate

Chol. – Cholesterol

DNA – Deoxyribose nucleic acid

FPG – Fasting plasma glucose

GAD₆₅ – Glutamic acid decarboxylase

GDM – Gestational diabetes mellitus

GHb – Glycohaemoglobin

HDL – High density lipoprotein

HFD – High fat diet

IAs – Insulin autoantibodies

ICAs – Islet cell antibodies

IDDM – Insulin dependent diabetes mellitus

IGT – Impaired glucose tolerance

IVGTT – Intravenous glucose tolerance test

LDL – Low density lipoprotein

MD – Maintenance diet

NCDs – Non-communicable diseases

NIDDM – Non-insulin dependent diabetes mellitus

OB/ IR – Obese/ Insulin resistant

OGTT – Oral glucose tolerance test

Pl. – Plasma

PPAR – Peroxisome proliferators activated receptor

RH – Rotary humidity

Rosi – Rosiglitazone

RXR – Retinoid X receptor

T1D – Type 1 diabetes

T2D – Type 2 diabetes

TAG – Tri-acyl glycerol

WHO – World Health Organisation

DECLARATION

Mr. K. B. Gabuza did the work presented here with the Medical Research Council's Diabetes Research Group under the supervision of *Dr. J. Louw* and *Prof. A. R. Opoku*. Work was done between January 2003 and December 2004. The document is submitted in partial fulfilment of a Masters degree in Biochemistry.

Mr Kwazi B. Gabuza

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ABSTRACT

Objective: To investigate anti-diabetic properties of the plant extract known as GUH in comparison to commercially available drugs metformin and rosiglitazone.

Methods: Male Wistar rats were fed a maintenance diet (MD) with tap water or a high fat diet (HFD) with sucrose solution for a period of twelve weeks. Thereafter a separate groups of animals were then treated with GUH at 5 different dose levels, metformin or rosiglitazone for twelve weeks. Food intake, liquid intake, weights, blood glucose, and insulin were monitored throughout the treatment period. An intravenous glucose tolerance test (IVGTT) was performed on representative animals from each treatment group prior to termination. At termination blood was taken and total cholesterol, triacylglycerol (TAG), low density lipoproteins LDL, and high density lipoproteins (HDL) were measured.

Results: The effect of GUH in MD fed rats was not marked. However, food intake and weight gain and total cholesterol were lower than in control animals. In HFD fed animals GUH, metformin and rosiglitazone had a significant effect. The extract reduced blood glucose and increased circulating insulin levels when compared to controls but results were not significantly different to metformin and rosiglitazone treated animals. As with metformin and rosiglitazone, GUH increased food intake with a concomitant weight increase. This weight increase was, nevertheless, less than with the other 2 treatments. In HFD fed animals GUH at the highest dose level increased the glucose clearance rate to a greater extent than metformin and rosiglitazone.

Conclusion: The results conclusively show that the extract GUH was at least as effective, and in some instances more effective, than currently used diabetes treatments. Although further work is required to investigate the mode of action, it is evident that

extracts of indigenous South African plants can be cost effective and efficacious treatments.

OVERVIEW

Non-communicable diseases (NCDs) are a problem in societies throughout the world. Many of these diseases have no cure, but they may be prevented or controlled. Diabetes is one of these and about 30 million people worldwide were reported to have diabetes in 1985. This number increased to 135 million in 1995 and in 2000 the World Health Organisation (WHO) estimated the number of people with diabetes to be 177 million (WHO, 2002). It is predicted that this will increase to at least 300 million by 2025 (Green, et al., 2003). Worldwide an estimated 4 million deaths per year are related to diabetes which contributes 9% to the global total. Most diabetics develop retinopathy, kidney failure, heart diseases, neuropathy, foot diseases and ketoacidosis, which cause deaths in the long term. According to WHO fact sheet N° 106, 1996 on NCDs in developing countries, 16% of South Africans suffer from hypertension and diabetes affects 4 to 15% of the South African population.

In 1997, Delpuech and Maire reported an increase in the number of overweight people and this can lead to obesity which is a risk factor for type 2 diabetes (T2D). Factors that have been associated with the development of diabetes are nutrition transition from frugal to lipid-rich diet especially when accompanied by reduced physical activity. There is a high prevalence of diabetes mellitus and impaired glucose tolerance (IGT) in urbanised South African blacks (Omar, et al. 1993). The association of T2D with urbanisation has important implications in view of large-scale urbanisation occurring in South Africa (Levitt, et al. 1993).

The cost of diabetes is extremely high. An estimated US\$ 44 billion was used for treatment of diabetes in the USA in 1997. Recent estimates of health care costs include those from Brazil (US\$ 3.9 billion), Argentina (US\$ 0.8 billion) and Mexico (US\$ 2.0 billion). The overall direct health care costs of diabetes range from 2.5 to 15% of a country's annual health care budget (WHO, 2002). This excludes the indirect cost, like reduced quality of life and ability to contribute to the community and the economy. Many people with diabetes experience reduced output at work due to vascular deterioration.

Vascular disease remains a major cause of morbidity and mortality in diabetes mellitus, in spite of recent improvements in outcome, some of which may be modulated by improved endothelial function (Andrew, et al., 2005). Apart from cardiac complications, the patients are subject to a wide range of vascular (i.e. peripheral vascular disease, stroke) and infectious complications. In the recent study, Tunceli, et al., (2005) showed evidence that diabetes affects patients, employers, and society not only by reducing employment but also by contributing to work loss and health-related work limitations for those who remain employed. This has an adverse effect on an already compromised economy. The emotional and social impact of diabetes and the demands of therapy may cause significant psychosocial dysfunction in patients and families (Committee Report, 2002). Depending on diabetes prevalence and the sophistication of the treatment available, Unwin, et al., (2001) estimated the economic growth in high prevalence countries of sub-Saharan Africa, such as South Africa and Botswana to be 17-20% lower than would be expected in the next ten years. Studies in India estimated that, for a low-income family with an adult with diabetes, as much as 25% of the family income may be devoted to diabetes care.

CHAPTER 1

INTRODUCTION

1.1 Diabetes Mellitus

Diabetes mellitus is a group of metabolic diseases or disorders characterised by the abnormal metabolism of fuels, especially glucose and fat. This leads to accumulation of glucose in the blood (hyperglycaemia). Hyperglycaemia results from defects in insulin secretion, insulin action or both (ADA Committee Report, 2000; Modern medicine, 2004). The chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels. Several pathogenic processes are involved in the development of diabetes. They include an autoimmune destruction of the β -cells of the pancreas, with consequent insulin deficiency, to abnormalities that result in resistance to insulin action. Insulin resistance is defined as a decreased response of peripheral tissues to insulin action (Xu, 2003) or a relative failure of insulin action on carbohydrate and lipid metabolism associated with hyperglycaemia causing a cluster of prevalent diseases. These include type 2 diabetes (T2D), obesity, dyslipidemias (particularly high triglycerides and low HDL-cholesterol levels), hypertension, and heart disease (Storlien, et al., 1997). Obesity and lack of physical activity are known to be major determinants, but evidence also suggested that dietary factors play a role in the development of T2D. A major characteristic of Western diets is a high intake of animal fat and meat (van Dam, et al., 2002).

Diabetes has been previously classified into five distinct types, i.e. insulin dependent diabetes mellitus (IDDM), non-insulin dependent diabetes mellitus (NIDDM), gestational diabetes mellitus (GDM), malnutrition related diabetes and other types

(Gabir et al., 2000). These types were characterised by either fasting hyperglycaemia or elevated levels of plasma glucose during an oral glucose tolerance test (OGTT). The American Diabetes Association Committee Report in 2002 included a new classification of impaired glucose tolerance in which plasma glucose during an OGTT was above normal but below those defined as diabetes. The ADA committee report also states that recent classification consist of three broad categories, i.e. type 1 diabetes, type 2 diabetes and gestational diabetes mellitus (GDM) based on aetiology. Current Medical Diagnosis and Treatment, (2005) states that the latest classification consists of type 1 diabetes, type 2 diabetes, and other types. Type 1 diabetes has two types that are immune mediated T1D and idiopathic T1D. Obese and non obese T2D are subtypes of type 2 diabetes. Other types of diabetes are maturity-onset diabetes of the young (MODY), diabetes due to mutant insulin, diabetes due to mutant insulin receptors, diabetes mellitus associated with a mutation of mitochondrial DNA and Wolfram syndrome.

1.1.1 Type 1 Diabetes (T1D)

T1D is immune mediated diabetes resulting from a cellular mediated auto-immune destruction of the β -cells of the pancreas (Atkinson, et al., 1994). Although it is an auto-immune disorder the aetiology of T1D remains unclear. Viruses have been implicated in the aetiology of T1D for the past several decades (Hyoty and Taylor, 2002). Viruses may attack and destroy the beta cells of the pancreas and directly cause diabetes with or without auto-immunity. Alternatively viruses may initiate or potentiate an auto-immune response against beta cells through molecular mimicry or autoimmune activation the latter of which results from the induction of inflammatory cytokines after infection (Rotter, et al., 1990). They are thought to act as inhibitors,

accelerators or precipitators of the disease and may function by direct or indirect mechanisms. The onset of T1D can occur at any age (Bach, 1994; Tisch and McDevitt, 1996) however it is mostly diagnosed in children. Ethnic variations in risk for populations residing in the same geographic area have been observed for T1D.

Markers of the immune destruction of the β -cells include islet cell autoantibodies (ICAs), autoantibodies to insulin (IAAs), autoantibodies to glutamic acid decarboxylase (GAD₆₅) and autoantibodies to the tyrosine phosphatases IA-2 and IA-2 β . One and usually more of these autoantibodies are present in 85-90% of individuals when fasting hyperglycaemia is initially detected. In T1D the rate of the beta cell destruction is quite variable, being rapid in some (mainly infants and children) and slow in others (mainly adults) (ADA Committee Report, 2002). Untreated T1D particularly in children and adolescents may lead to development of ketoacidosis as the first manifestation of the disease. Diabetic ketoacidosis is a complication of diabetes mellitus caused by build up of by-products of fat (ketones), which when glucose is not available as a fuel source the body. In ketoacidosis ketones build up in the blood and spill over into the urine and blood becomes more acidic than body tissues leading to damage in body tissues. Others, particularly adults, may retain residual β -cell function sufficient to prevent ketoacidosis for many years. This form of type 1 diabetes eventually becomes dependent on insulin for individual survival and individuals are at risk for ketoacidosis. Ketoacidosis results from little or no insulin secretion as manifested by low or undetectable levels of plasma c-peptide. Some patients with this form of diabetes have insulinopenia and are prone to ketoacidosis, but have no autoimmunity. Studies show that a minority of patients with type 1 diabetes fall into this category and of those who do most are of African or Asian

origin. This form of diabetes is strongly inherited and lacks immunological evidence for β -cell autoimmunity. An absolute requirement for insulin replacement therapy in affected patients may come and go. Drugs such as alloxan, streptozotocin, etc lead to so-called drug and chemical-induced diabetes and damage of β -cells very similar to T1D.

1.1.2 Type 2 Diabetes (T2D)

T2D is a disease in which insulin resistance and usually relative, rather than absolute, insulin deficiency occurs. At least initially, and often throughout their lifetime, these individuals do not need insulin treatment to survive (ADA, 2002). Type 2 diabetes is a heterogeneous disease with complicated pathogenesis which is related to the genetic susceptibility and life style, especially dietetic style (Zhang et al., 2003). Individuals with insulin resistance, which is defined as a decreased response of the peripheral tissues to insulin action, are predisposed to developing T2D. Increasingly insulin resistance has been recognised as an integral feature of the so called metabolic syndrome, which includes glucose intolerance, insulin resistance, obesity, hypertriglyceridemia, low HDL cholesterol, hypertension and accelerated atherosclerosis (Xu, et al., 2003). Both hyperinsulinaemia, a marker for insulin resistance, and obesity are established risk factors for T2D (Carnethon, et al., 2002).

The capacity to produce insulin is determined by the total β -cell number and β -cell functional activity. The β -cell mass is capable of long term adaptation by increasing the β -cell number through hyperplasia and neogenesis (Maedler, et al., 2001). Hyperinsulinaemia has long been recognised as a feature of obesity-related insulin

resistance in man and in rodents but with obscure mechanism (Milburn, et al., 1995). Insulin resistance is often associated with increased body weight. Recently, various adipocyte-secreted proteins have been described which are altered in obesity, affect insulin sensitivity and might, therefore provide a link between obesity and insulin resistance (Fasshauer and Paschke, 2003). Western diets, which are characterised by a high intake of animal fat and meat, have been indicated to be strongly associated with T2D.

1.2 Insulin

Insulin is the hormone that stimulates the synthesis of glycogen. The B cells of the Islets of Langerhans are able to modulate both the production and the release of insulin in response to changing metabolic demands. Insulin results from the precursor proinsulin consisting of signal peptide, A, B, and C peptide chains. Proinsulin is discharged into the cisternal space of the rough endoplasmic reticulum, where proteolytic enzymes immediately cleave it into proinsulin, removing the signal peptides. Proinsulin is the peptide containing A and B chains of insulin joined by the C peptide. Proinsulin is then transported by microvesicles to the Golgi apparatus, where it is packaged in vesicles that are enclosed by a membrane containing an ATP-dependent proton pump. The conversion of proinsulin to insulin is initiated in the Golgi complex and continues within the maturing secretory granules through the sequential action of prohormone convertase (2 and 3) and carboxy peptidase. Together these enzymes act to remove the C peptide chain, liberating two cleavage dipeptides and finally yielding insulin (Pickup and Williams, 1997).

Secretion of insulin is a response to increased glucose in the blood. When blood glucose levels rise (e.g. after a meal), insulin is secreted from the pancreas into the

pancreatic vein, which empties into the portal vein system, so that insulin traverses the liver before it enters the systemic blood supply. Insulin acts rapidly to lower blood glucose concentration in several ways. Glycogen synthesis stimulation and glycolysis (glucose breakdown) inhibition in liver and muscles is the action of insulin. There are several other physiological effects of insulin that also serve to lower blood and tissue glucose levels. Insulin stimulates the active transport of glucose (and amino acids) across the plasma membranes of muscle and adipose tissue. This is a short term effect. It also increases cellular utilisation of glucose by inducing the synthesis of several important glycolytic enzymes, namely, glucokinase, phosphofructokinase, and pyruvate kinase. In addition, insulin acts to inhibit several enzymes of gluconeogenesis which is a long term effect. These various actions enable the organism to respond quickly to an increase in blood glucose levels (Randle, et al., 1963).

1.3 Insulin resistance

Insulin resistance is a reduced sensitivity of tissue to the action of insulin. When insulin resistance occurs the body attempts to overcome this resistance by secreting more insulin. This compensatory state of hyperinsulinemia (high insulin levels in the blood) is considered to be a marker for the insulin resistance syndrome (Storlien, et al., 1997).

Insulin resistance syndrome is the combination of abnormalities in glucose and lipid metabolism, obesity, and high blood pressure in the same individual. It is also called Syndrome X or Deadly Quartet. The development of T2D occurs when the pancreas fails to sustain this increase in insulin secretion (Nathan, 1993). It is not understood

how insulin resistance contributes to the presence of high blood pressure but it is known that the high insulin levels resulting from insulin resistance contribute to abnormalities in blood lipids (Goldstein, 2003).

1.4 Diagnosis and monitoring of diabetes

Diagnosis of diabetes primarily depends upon the results of specific glucose tests. These glucose tests are just part of the information that goes into the diagnosis of diabetes. The diagnostic criteria for diabetes mellitus are impaired fasting glucose and impaired glucose tolerance. The oral glucose tolerance test and fasting plasma glucose level are convenient tests widely used for diagnosis because of their ease of administration, low cost and patient acceptability (Rohl, 2004). It has been suggested that testing for diabetes should be considered in all individuals aged 55 years and above, and three-yearly interval tests should be repeated if the results are normal (ADA, 2000). Testing of people of younger age who have impaired glucose tolerance or impaired fasting plasma glucose, and who fall in certain high risk non-Caucasian groups aged 35 or over should be considered (ADA, 2001). A frequent testing of people aged 45 years or over with obesity, hypertension or who are a first degree relative with a T2D should be done (HDS Study Group, 1993). All people with clinical cardiovascular disease, women with previous gestational diabetes and obese women with polycystic ovary syndrome should be tested frequently. Although only two tests have been mentioned above for diagnosis of diabetes there are other tests that can be done in a laboratory for diagnosis of diabetes. These tests include intravenous glucose tolerance test, glycated haemoglobin, glucose challenge test, lipid panel, two hour post prandial glucose and glucose clamping which are explained in details in sections 1.4.1 to 1.4.8. All of these tests should be used one after the other in

order to confirm the results. This means there is no one test, which can be used on its own to diagnose a person as a diabetic.

1.4.1 Fasting Plasma Glucose (FPG) Test

The blood glucose levels before and after a meal vary. Studies have been done in order to determine the expected level limits before and after a meal. Fasting glucose level is used to determine a person's blood glucose level after an 8 to 12 hour fast. It may be used to diagnose pre-diabetes and diabetes. It is also used for people with diabetes so as to monitor the disease. The normal fasting glucose is < 6 mmol/l. Individuals with a FPG ≥ 6.1 mmol/l, but < 7.0 mmol/l are said to have impaired fasting glucose. A FPG, which is ≥ 7.0 mmol/l, leads to the provisional diagnosis of diabetes. A FPG can be used as a tool in epidemiological studies for estimates of diabetes prevalence and incidence as was recommended in the interests of standardising the fieldwork. This came about due to the impossibility of doing an oral glucose tolerance test in some places and for the excessive time that it takes (ADA, 2002), but tests like OGTT must be performed as confirmation tests.

1.4.2 Oral Glucose Tolerance Test (OGTT)

OGTT is a test that is used to diagnose pre-diabetes and diabetes. It is performed after an overnight fast. A blood sample is taken and then the patient drinks a 75g anhydrous glucose to total liquid volume of 300-350 ml (WHO, 1999). Blood samples are taken at intervals at 0, 30 minutes and hourly for 3 hours. Test results are compared with a standard to show how the body metabolises glucose over time. For the test to give reliable results a person to be tested must be in good health, normally active and take

no medicine as this may affect the results (ADA, 2002). In a person without diabetes the 2-hour glucose level is less than 7.8 mmol/l and all values between 0 and 2 hours are less than 11.1 mmol/l. A person is said to have impaired glucose tolerance when the fasting plasma glucose is less than 7.8 mmol/l, the 2 hour glucose level is between 7.8 mmol/l and 11.1 mmol/l, and at least one of the values between 0 and 2 hours is more than 11.1 mmol/l. Diabetes is diagnosed when two glucose tolerance tests show that the blood glucose level at 2 hours is more than 11.1 mmol/l and so is at least one of the glucose levels taken between 0 and 2 hours (ADA, 2002).

1.4.3 Glucose Challenge Test (GCT)

GCT is also known as the 50-gram glucose challenge or one-hour glucose screening test. It is a screening test that measures blood glucose levels one hour after 50 grams of glucose has been consumed (Ford-Martin, 2004). GCT is used for screening of gestational diabetes in pregnant women who are considered to be at risk. The test can be performed once and if elevated results show an OGTT should follow. Normal values for GCT are 7.8 mmol/l or less. A glucose threshold value > 7.8 mmol/l means that gestational diabetes is a possibility (Tan, et al., 2001)

1.4.4 Two-hour postprandial glucose

Two-hour postprandial plasma glucose is also called postprandial plasma glucose test. This test measures the body's ability to metabolise carbohydrates and produce insulin. The test is performed to screen for diabetes or confirm results from the fasting plasma glucose test. The test is also used to evaluate the effectiveness of medication or dietary therapy in diagnosed diabetics. Generally, levels of less than 8.1 mmol/l are

considered normal when using the glucose oxidase or hexokinase laboratory methods (Edelman, et al., 2002). Two-hour postprandial glucose values of 11.1 mmol/l or higher indicate diabetes. Further laboratory tests may be required to confirm this diagnosis.

1.4.5 Intravenous Glucose Tolerance Test (IVGTT)

This test is not normally used for diagnosis but is used in the investigation of early insulin secretion abnormalities in prediabetic states. When glucose is given intravenously it is termed an intravenous glucose tolerance test (IVGTT). The results determine the insulin action and glucose metabolism (Vicini, et al., 1999).

1.4.6 Glycohaemoglobin (GHb) Test

A glycohaemoglobin test gives an indication about chronic hyperglycaemia and it reflects the patient's metabolic control (Herman et al., 2000). It is generally used along with other glucose blood tests to monitor the treatment of diabetes and to determine whether, on average, a patient's blood glucose levels are high. High glycohaemoglobin levels have been found to correlate with the onset of several side effects experienced by diabetic patients including nerve damage, kidney disease and loss of eyesight. Most people with diabetes will have a glycohaemoglobin test between one and four times a year. No changes in diet, activity or daily medications are required before testing. Three types of glycohaemoglobin, A_{1a}, A_{1b}, A_{1c} are measured in a total glycohaemoglobin test. Normally, only a small percentage of haemoglobin in the blood (4% to 6%) has glucose bound to it. However, people with diabetes (or other conditions that increase their blood glucose levels) have more

glycohaemoglobin than normal (Spengler, 2002). The results reflect the amount of glycohaemoglobin multiplied by 100 to produce a percentage. Usually only the glycohaemoglobin A_{1c} and total glycohaemoglobin values are reported. ADA recommends that people with diabetes have a total glycohaemoglobin less than 7%. If levels are greater than 8%, the ADA recommends that diabetes treatment be re-evaluated (ADA, 2002). A total glycohaemoglobin A_{1c} level in excess of 9% means that a person's diabetes has been poorly controlled during the previous 2 to 3 months.

1.4.7 Glucose Clamp Technique

The glucose clamp is regarded as the “gold standard” method for the determination of the action profiles of blood glucose lowering agents (Thome-Duret, et al., 1996). Both insulin sensitivity and the degree of blood glucose lowering effect of a drug substance can be exactly quantified by applying the glucose clamp technique. In the automated technique a patient's blood glucose is measured continuously i.e. every minute while, by means of a variable intravenous glucose infusion, a defined glucose level is held constant. The amount of infused glucose reflects the metabolic activity of a given anti-diabetic drug over time. It can be performed manually by taking blood samples at regular intervals, measuring the blood glucose level and then appropriately adapting the glucose infusion rate. This method however, requires numerous skilled personnel (Heinemann and Koschisky, 2002). Further it is exact and reliable only to a limited extent due to much error in application. The use of automated techniques in which plasma glucose levels are measured continuously and glucose infusion rates adjusted every minute reduces the need for experienced personnel. Even rapid and subtle changes in glucose requirements, as they occur, for instance with rapid acting insulin analogues, can be rapidly and adequately adjusted.

1.4.8 Lipid Panel (Profile)

A lipid panel is a group of tests that are often ordered together to determine risk of coronary disease. The lipid profile includes total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein cholesterol and triglycerides. The total cholesterol equals the sum of VLDL, LDL and HDL in a formula $\text{Total cholesterol} = \text{HDL cholesterol} + \text{VLDL cholesterol} + \text{LDL cholesterol}$. Most clinical laboratories measure the total cholesterol, total triglycerides and the amount of cholesterol found in HDL fraction which is easily precipitated. $\text{VLDL} = \text{Triglycerides}/5$ is used to calculate VLDL since most triglyceride is found in VLDL particles which contains five times as much triglycerides by weight as cholesterol. This is used in fasting samples and it works only when the triglyceride level is less than 400-500 mg/dL. When triglycerides are high, LDL and VLDL cholesterol levels can be determined after ultra centrifugation or by direct chemical measurement. Although total cholesterol is stable over time, HDL and triglyceride measurements may vary considerably because of analytical error in the laboratory and biological variations in a patient's lipid level. It is thus recommended that the LDL should always be estimated as the mean of at least two determinations; if these two estimates differ by more than 10% a third lipid profile is obtained and is estimated as follows: $\text{LDL cholesterol} = \text{Total cholesterol(mg/dL)} - \text{HDL cholesterol(mg/dL)} - \text{Triglycerides(mmol/L)}/2.2$ this test is also called lipid profile.

1.5 Prevention and treatment of diabetes

Type 2 diabetes has long been linked with behavioural and environmental factors such as overweight, physical inactivity, and dietary habits. Several treatments are effective in preventing complications of diabetes, but these are sub-optimally used, and the disease itself is chronic, progressive, and degenerative (Narayan, et al., 2001). Currently, it is recommended that overweight individuals undertake moderate weight loss combined with increased physical activity to lessen risks such as obesity which contribute to insulin resistance and so reduce the risk of developing type 2 diabetes (Goodpaster, et al., 2003). Exercise, diet and weight control may be insufficient or patient compliance difficult, rendering conventional drug therapies (oral glucose lowering agents and insulin injections) necessary in many patients. In addition to adverse effects, drug treatments are not always satisfactory in maintaining euglycaemia and avoiding late stage diabetic complications. As an alternative approach, diabetic patients and health care professionals increasingly seek medicinal herbs with antihyperglycaemic activities (Dey, et al., 2002).

1.5.1 Exercise, diet and weight management

Appropriate use of diet and exercise can improve insulin sensitivity and glycaemic control and decrease the need for oral medications or insulin (Nelson, et al., 2002). Although there is some controversy over the optimal diet for adults with T2D there is a consensus to increase consumption of fruits and vegetables and decrease daily consumption of saturated fats (ADA, 2002). Regular exercise alone can slow the development of type 2 diabetes, and a regular walking program was shown to reduce total mortality by 42% in patients with T2D (Zarich, 2003). Physically trained

individuals typically manifest both a high degree of insulin sensitivity for glucose disposal and a high reliance upon fat oxidation by skeletal muscle during physical activity. It has also been demonstrated that acute exercise enhances insulin stimulated glucose uptake more in trained than untrained muscle, suggesting the importance of regular exercise (Lim, et al., 2004). Studies have shown exercise to result in improvement of low-density lipoprotein (LDL) sub-fractions, with a decrease in small dense particles, and may therefore protect against coronary heart disease (CHD) despite a lack of reduction of total or LDL cholesterol. Diet plays a role in the control of metabolism. The phenolics are primarily found in foods of plant origin, such as fruits, vegetables, legumes, coffee, tea and wine (Maga, 1978). Chlorogenic acid and caffeic acid are antioxidants *in vitro* and might therefore contribute to the prevention of cardiovascular diseases. Chlorogenic acid has been proven in animal studies *in vitro* to inhibit hydrolysis of glucose-6-phosphate enzyme in an irreversible fashion. This mechanism allows chlorogenic acid to reduce hepatic glycogenolysis (breakdown of glycogen to glucose) and to reduce absorption of new glucose. *In vivo* studies demonstrated that administration of chlorogenic acid lessens hyperglycaemic peak resulting from glycogenolysis brought about by administration of glucagon, hyperglycaemiant hormone. Some studies also confirmed a reduction in blood glucose levels and an increase in the intrahepatic concentrations of glucose-6-phosphate and of glycogen. High levels of tannins have been found in cereal grains such as sorghum and sunflower seeds, which contain considerable amounts of chlorogenic acid (Takii, et al., 1997; Welsch, et al., 1989).

1.5.2 Oral hypoglycaemic agents

The oral hypoglycaemic drugs have long been used for the control of raised blood glucose levels. Since the most important pathological process during the development of diabetes involves three key organs, which are the pancreatic islets, liver and skeletal muscles, almost all anti-diabetic therapies are aimed at these organs (Dey, et al., 2002). These drugs aim to correct insulin resistance and secretion of insulin. Currently there are five available classes of oral hypoglycaemic agents, each displaying unique pharmacological property. These classes are sulfonylureas, meglitinides, alpha glucosidase inhibitors, thiazolidinediones and biguanides (Luna and Feinglos, 2001). The mode of action for each group is explained below, with more information on metformin and rosiglitazone, which were used in this study.

1.5.2.1 Sulfonylureas

Sulfonylureas enhance insulin output by the pancreas. They exert their insulinotropic effects by closing ATP sensitive potassium channels in B cell membranes which results in depolarisation of the membrane. This in turn promotes influx of calcium, and the rise in intracellular calcium stimulates insulin secretion. Sulfonylureas bind to the receptor-like structures on the B cell, which may be closely linked to, or form an integral part of, the potassium channels. The binding capacity of different sulfonylureas for these receptors closely parallels their ability to stimulate insulin secretion. Clinical NIDDM is characterised by loss of first phase insulin secretion and impairment of the second phase, although only the first phase insulin secretion is impaired early in the disease. Most studies have been unable to demonstrate any appreciable effect of sulfonylureas on first-phase insulin secretion in patients with

established NIDDM, but the second phase is enhanced (Pickup and Williams, 1997). Drugs of this type may cause hypos (decrease in blood glucose levels) and also stimulate weight gain, because a lot of insulin produced increases the energy uptake and creates a positive energy balance. Drugs with shorter half-life are preferable. Sulfonylureas are often used with Metformin if the patient is overweight.

1.5.2.2 Meglitinides

Non-sulfonylureal insulin secretagogues, also known as the meglitinides, lower blood sugar levels by increasing the release of insulin from the pancreas (Dornhorst, 2001). Because these drugs cause only small amounts of insulin to be released when glucose is not present they must be given with meals. Meglitinides may be used alone or in combination with metformin. Typical examples of drugs in this class are Nateglinide (Starlix) and Repaglinide (Prandin).

1.5.2.3 Alpha glucosidase inhibitors

Drugs in this category include acarbose and miglitol. They are competitive inhibitors of the alpha glucosidase, enzymes that are responsible for the breakdown of complex carbohydrate into their monosaccharide components ready for absorption. The complex carbohydrate is therefore absorbed further along the small intestine, leading to a slower rise in postprandial blood glucose level and improving postprandial glycaemic control. The drug can be used either alone or in combination with other diabetic therapies including insulin, and may reduce glycated haemoglobin (HbA1c) by up to 1% over a period of months. However carbohydrate load that enters the large bowel however often leads to episodes of diarrhoea.

1.5.2.4 Biguanides

Biguanides reduce the amount of glucose the liver releases into the blood stream. Metformin is an example of the drugs in this category. It reduces plasma glucose concentrations by an average of 25% with a somewhat better effect on postprandial than on fasting plasma glucose levels. There is no single clear explanation on the mechanism of the drug in reducing blood glucose, but various mechanisms have been proposed. The reduction in postprandial plasma glucose concentration could be due to increased peripheral glucose uptake, enhanced suppression of hepatic glucose production, or both. Various studies have reported a 20-25% increase in peripheral glucose uptake during treatment with metformin, mostly due to an increase in non-oxidative glucose metabolism. One study suggested that this effect can be due to reversal of the fatty acid formation from glucose by a primary inhibitory effect on lipid oxidation, leading to compensatory increase in glucose oxidation (Pickup and Williams, 1997).

Recent studies in humans suggest that the main metabolic effects of metformin in patients with T2D are to inhibit gluconeogenesis and decrease hepatic glucose output, with little effect on peripheral glucose uptake. In patients with IGT, it acts through enhanced glucose uptake. Zhou et al., (2001) reported that the increased phosphorylation and activation of AMP-activated protein kinase leads to the effects on glucose and lipid metabolism. This is brought by the phosphorylation and inactivation of acetyl CoA carboxylase, as a result of AMPK activation, which serves to inhibit the proximal and rate limiting step of lipogenesis as shown in figure 1.1. It can cause diarrhea and should not be used by anyone with impaired kidney function or other severe medical illnesses.

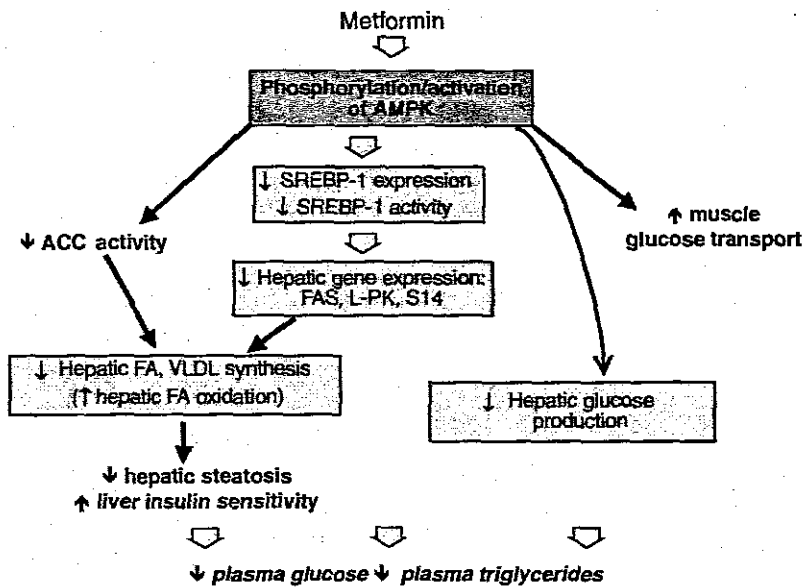


Figure 1.1: Model for the mechanism by which metformin mediates effects on lipids and glucose metabolism. FA, fatty acids (taken from Journal of Clinical Investigation Volume 108: 1173)

1.2.5.5 Thiazolidinediones

Thiazolidinediones reduce insulin resistance in the muscle and liver and thereby improve cells' responsiveness to insulin. The drugs in this category include troglitazone, rosiglitazone and pioglitazone. These drugs are also called glitazones. They activate peroxisome proliferator activated receptor (PPAR) gamma, in the cell nucleus by binding to it. After binding to the glitazone, PPAR hooks up with another receptor protein called retinoid X receptor (RXR). The glitazone PPAR-RXR complex then latches on to the DNA within the nucleus to quickly turn on a number of genes controlling a series of biochemical reactions. This results in the enhancement of a number of metabolic processes. The end result is greater insulin sensitivity and high density lipoprotein (HDL) cholesterol production, and lower triglyceride and blood glucose levels (Battacharyya, 2001). Troglitazone has been withdrawn from the

market after the reports of liver failure in some people who were taking it. But liver failure has not yet been recognized with the other two drugs in this class.

1.6 The use of herbal and spiritual healing for diabetes

Alternative treatments for diabetes have become increasingly popular during the last several years (Sinha, et al., 1996). Many conventional drugs have been derived from *phytotypic molecules in medicinal plants*. Metformin is an example of an efficient glucose-lowering agent. The development of metformin, an efficient glucose lowering agent was based on the use of *Galega officinalis* to treat diabetes (Bailey and Day, 1989). Since then the research on medicinal plants has increased especially where considerable work has been done in this field. China and India have a lot of work done in this field of research. More than 400 traditional plant treatments for diabetes mellitus have been reported although only a small number have received scientific and medical evaluation to assess their efficacy (Dey, et al., 2002).

Materials that are able to manipulate glucose absorption from the small intestines are widely found in plants (Matsumoto, et. al., 1993). Phenolics are widespread in nature and are primarily found in foods of plant origin, such as fruits, vegetables and legumes, and coffee, tea and wine (Maga, et al., 1978). The interaction between plant polyphenols and proteins has been well known from early times (Welsch, et al., 1989). Polyphenols enhance the insulin action and hence metabolism. The Indian medicinal plant *Gymnema sylvestre* has been reported to control the rise in blood glucose. *Gymnema sylvestre* is an Indian herb used in Ayuverda, the ancient Hindu medicine system of India, primarily used for T2D and it continues to be recommended today. Research has shown that its extracts from leaves raised insulin levels in healthy

volunteers which could possibly be due to regeneration of β -cells in the pancreas (Joffe, 2001).

The impact of herbal medicines is starting to be recognised worldwide. Internationally a wide range of herbs has been studied. Western herbs that have been recommended to treat diabetes include bitter melon (*Mormodica charantia*), (Qixuan, et al., 2003) *Allium cepa* (onion), *Allium sativum* (garlic), bilberry, Aloe vera and salt bush to mention a few. In India broad research has been done on a number of plants and the plants that appear in their scientific reports include Fenugreek, *Gymnema sylvestre*, and *Pterocarpus Marsupium* for example. The Chinese have a wide range of plants that they recommend for treatment of diabetes. Scientific reports include *Ginseng*, Green tea, Quei Fu Di Huang Wan, Ruo Gui or Fu Zi and Tang Niao Kang, to list some known as being efficacious.

Research in Africa has focussed little on traditional medicine over the years. There are numerous plants that traditional healers utilise to treat diabetes, but there is a lack of written information on them. Herbal teas have been used for treatment of diabetes for many years. The study which was done by Ferreira, et al., (1998) showed that phenolic compounds are contained in honey bush tea (*Cyclopia intermedia*). As has been previously mentioned phenolics possess a protein binding ability, which enhances the functioning of the proteins. Peltzer, et al., 2001 showed in their study that about 93% of the users of traditional medicine in the Northern Province, South Africa have faith in traditional healing. There has been increased research on traditional medicines worldwide after the WHO expert committee recommended further investigation of traditional medicinal herbs.

In South Africa there is a considerable change in the health system pertaining to the recognition of traditional healing. The study by Chifakacha, (1994) has shown that the traditional healer is the first and nearest contact for the rural black African. This makes traditional healers form the primary health care for such Africans. However this is inadequate for patient needs and a link between traditional healers and the western health service is required. Studies that were performed in most parts of Africa show that an emergent urban Black has generated an informal model of treatment that depends on both Western and traditional care (Farrand, 1984). Most of the people who use traditional medicine for self-medication are not referred to in the literature which has a large effect in the limited information on traditional healing (Cocks and Dold, 2000). In the study by Singh, et al., 2004 on complementary alternative medicine (CAM) that took place in Chatsworth, South Africa 79.2% of CAM users benefited from it while 11.7% had no improvement in their illnesses and 9.7% were not sure if there were any beneficial effects at all. This study showed almost the same response to the study by Puckree, et al., (2002), in Durban, South Africa which showed 70% of the patients preferred to consult traditional healers as their first choice.

There is an extensive list of plants that have been collected from S.A. traditional healers that are of use when treating diabetes. Although the mechanism of action of these plants cannot as yet be explained, examples of such plants will be listed. They include *Terminalia planerophletin*, *Terminalia sericea*, *Mormodica balsamina*, *Mormodica foetida*, *Catharanthus roseus*, *Brachylaena elliptica*, *Artemisia afra*, *Cannabis Sativa*, *Sutherlandia* and many more.

In this study the plant identified as GUH is used to examine its anti-diabetic properties. The efficacy of the plant is determined by considering its ability in controlling some parameters involved in metabolism. The comparison is made between the GUH and the other widely used hypoglycaemic drugs, namely metformin and rosiglitazone.

CHAPTER 2

MATERIALS AND METHODS

2.1 Animals

Wistar rats were bred in the Diabetes Group's Animal Unit of the MRC. They were fed maintenance diet and water. The maintenance diet has been used for breeding and maintaining rats successfully since 1986. They were maintained under a 12 hour (6:00-18:00) light dark cycle at a temperature of $23\pm 4^{\circ}\text{C}$. Three weeks after birth males were randomly divided into fourteen groups each consisting of ten rats. They were marked with non-toxic food colorant and placed in groups of three in a cage. They were weighed and their weights recorded for baseline weight measurements. The weights were recorded weekly thereafter, throughout the experiment period.

2.2 Treatment groups

2.2.1 Obese (Insulin resistant) animals

At weaning (three weeks) eight groups were weighed for baseline as in 2.4. They were fed *ad libitum* a high fat diet which induces obesity and leads to insulin resistance (Nkabinde, 2003) and 30% sucrose solution for twelve weeks. After twelve weeks blood was collected as in 2.9.2 for baseline insulin measurement. Sucrose was substituted with GUH plant extract in five groups. One group remained on HFD and sucrose. The other two groups received metformin and rosiglitazone instead of sucrose and the diet remained the same. The food and liquid intake was monitored as in 2.7. The blood glucose was also monitored every four weeks throughout the experiment, starting from four weeks after weaning. After twenty-four weeks run on

the experiment three rats in each group were tested for glucose tolerance by intravenous glucose tolerance test (IVGTT) as in 2.9.4. After IVGTT animals were euthanased and tissues were collected and frozen. The remaining seven were euthanased and pancreas, liver, kidney were fixed in 10% buffered formalin pH 7.0 [formalin 10ml, NaH_2PO_4 0.4g, Na_2HPO_4 0.65g, distilled H_2O to 100ml].

2.2.2 Lean animals

Six groups were fed maintenance diet and tap water. Pellets were weighed before feeding and again the following day to determine food intake. After twelve weeks water was substituted with GUH for five groups and one group remained on tap water. The procedures performed in this group were the same as those performed in the obese animals in 2.2.1 above.

2.3 Diet

High fat diet (HFD) prepared in the Primate Unit's Kitchen and maintenance diet (MD) was purchased from Epol (Pretoria). The diet composition for both the diet is shown in Table 2.1

Table 2.1: Diet composition

Diet	HFD	MD
Protein	15%	22%
Fats	40%	6%
Carbohydrates	45%	72%

Table 2.2: Treatment of OB/IR model through study period

Groups	C1	C2	C3	C4	C5
Time (Months)					
1	HFD + 30% sucrose	HFD + 30% sucrose	HFD + 30% sucrose	HFD + 30% sucrose	HFD + 30% sucrose
2	HFD + 30% sucrose	HFD + 30% sucrose	HFD + 30% sucrose	HFD + 30% sucrose	HFD + 30% sucrose
3	HFD + 30% sucrose	HFD + 30% sucrose	HFD + 30% sucrose	HFD + 30% sucrose	HFD + 30% sucrose
4	HFD extract +	HFD extract +	HFD extract +	HFD extract +	HFD extract +
5	HFD extract +	HFD extract +	HFD extract +	HFD extract +	HFD extract +
6	HFD extract +	HFD extract +	HFD extract +	HFD extract +	HFD extract +

Other OB/ IR treatment groups were treated the same way as the group above except for week 4-5 where instead of extract one group was given either *rosiglitazone*, sucrose or metformin. The difference between untreated and the control group was the diet and liquid given. The control group was given water and MD while untreated group was given 30 % sucrose and HFD. These are the groups that were left without treatment.

Table 2.3: Lean animals through treatment period

Group	Control	C1 treated Lean	C2 treated Lean	C3 treated Lean	C4 treated Lean	C5 treated Lean
Time						
Month 1	MD + Tap H ₂ O	MD + Tap H ₂ O	MD + Tap H ₂ O	MD + Tap H ₂ O	MD + Tap H ₂ O	MD + Tap H ₂ O
Month 2	MD + Tap H ₂ O	MD + Tap H ₂ O	MD + Tap H ₂ O	MD + Tap H ₂ O	MD + Tap H ₂ O	MD + Tap H ₂ O
Month 3	MD + Tap H ₂ O	MD + Tap H ₂ O	MD + Tap H ₂ O	MD + Tap H ₂ O	MD + Tap H ₂ O	MD + Tap H ₂ O
Month 4	MD + Tap H ₂ O	MD extract +	MD extract +	MD extract +	MD extract +	MD extract +
Month 5	MD + Tap H ₂ O	MD extract +	MD extract +	MD extract +	MD extract +	MD extract +
Month 6	MD + Tap H ₂ O	MD extract +	MD extract +	MD extract +	MD extract +	MD extract +

2.4 Body weight

In all the treatment groups, (at 09h00) weight was measured and recorded at baseline and weekly throughout the experiment.

2.5 Preparation of extracts (GUH)

A large quantity of plant material was harvested from natural or cultivated plants to ensure that the same plant material was available for all the tests. The plant material was dried to less than 10% moisture content by forced air circulation at 45°C and 30% relative humidity (RH), whereafter it was pulverised with Retch rotary mill. Tap water (100 kg) at 95-98°C was added to 10 kg of pulverised plant material and continuously stirred for 30 minutes (final extract at 70°C). The extract was centrifuged at 8000g for 15 minutes to remove insoluble matter. It was cooled to room temperature with heat exchanger. The undiluted extract was frozen at -20°C. The extract was diluted to different concentrations by adding tap water to undiluted extract.

2.6 Administration of drugs

2.6.1 Administration of metformin

Metformin hydrochloride (850mg, Rolab, Johannesburg) was dissolved in 85 ml of distilled water. Dissolved metformin was given with 30 ml of water each day. This was enough for one day for each rat. The dosage was calculated to be 10 mg/ml in distilled water and rats were given 2µl per gram body weight which is equivalent to 0.02 mg/g body weight. This is double the dosage of humans of 850 mg/day.

Dosage calculation:

For an individual weighing 75 kg = 850 mg/ 75 kg

= 11.3 mg/kg

= 0.01 mg/g body weight

2.6.2 Administration of rosiglitazone

Avandia rosiglitazone maleate (GlaxoSmithKline, 4 mg, Bryanston, South Africa) was dissolved in 1 ml acid phosphate buffer with pH of 2.3. The dissolved tablet was given with 30 ml water in a dosage of 4 mg/kg body weight. The medication was given daily throughout the treatment period except on days where the rats have to be starved for blood collection.

2.6.3 Dosage of GUH

The GUH was given in 4, 8, 12, 15 and 20% amounts that were coded C1, C2, C3, C4 and C5 respectively. The amount of GUH extract given to each animal was calculated by the amount of liquid taken divided by the total amount of extract multiplied by the concentration. That was worked out using the formula:

Actual dosage = (liquid intake/ total liquid given) X the concentration of an extract

The dosage was calculated as g/kg weight for each animal and therefore the dosage for the entire group was calculated. This was used to plot XY charts to show the effect of dosage to weight gain and food intake. The formula used was:

Dosage = (Liquid intake/ 100) X initial concentration

2.7 Food and liquid intake

The amount of food and liquid consumed was monitored throughout the treatment period in all groups. Every morning at the same time food was weighed and liquid measured before giving to animals. The following morning the food left was weighed and recorded. The liquid was measured every two days during the week and after three days during weekend. The average intake was calculated as average of food or liquid taken in per week. Intake was recorded as the difference between the quantity given and the remaining volume.

2.8 Intravenous injection

Animals were anaesthetised by inhalation of 98% oxygen and 2% fluothane (AstraZeneca Pharmaceuticals). An incision was made on the side of the tail to expose the vein. A cannulated needle was inserted in the vein under a stereomicroscope (Wild Heerbrugg Plan). The substance was injected through cannula Abbocath (Abbott Laboratories).

2.9 Blood analysis

2.9.1 Fasting plasma glucose

Animals were fasted overnight. The tip of the tail was cut using a pair of scissors and a drop of blood was used to determine the glucose level using a glucometer Precision Q.I.D (Abbott Laboratories).

2.9.2 Blood collection

Animals were anaesthetised by inhalation of 98% oxygen and 2% fluothane (AstraZeneca Pharmaceuticals). The tail tip was amputated and blood collected into Epindorff tubes and stored on ice. Thereafter it was centrifuged at 2500 r.p.m for 15 minutes. Using micropipette 50µl aliquots were placed in Nunc tubes and stored at minus 80°C until used.

2.9.3 Serum for insulin and lipids

Insulin was measured at baseline or before treatment for all the treatment groups. For obese and lean animals it was continued monthly during treatment. Samples were taken for insulin measurement by radioimmunoassay using ¹²⁵I-labelled human insulin as tracer and rat insulin as standard (Linco Research, St. Charles, MO. USA). The lipid profile was done using an autoanalyzer (Technicon RA 1000).

2.9.4 Intravenous Glucose Tolerance Test (IVGTT)

Animals were anaesthetised by inhalation of 98% oxygen and 2% fluothane (AstraZeneca Pharmaceuticals). The tip of the tail was amputated and baseline glucose was measured and recorded. Intravenous injection of 50% glucose at a dose of 0.5 mg/kg was performed as in 2.8 over 20 seconds. Blood glucose measurements were done at 1, 2, 3, 5, 10, 20, 30, 40, 50 and 60 minutes. Thereafter the rats were euthanased.

2.10 Termination procedures

After IVGTT was performed animals were euthanased by injecting them with 6% pentobarbitone (AstraZeneca, Johannesburg). A laparotomy incision was made and blood was collected using a syringe. Collected blood was stored on ice in heparin tubes. The blood was centrifuged and aliquots were used for blood chemistry. Liver, pancreas, abdominal fat and muscle were collected and snap frozen in liquid nitrogen. Tissues were stored at -80°C until use. The remaining animals that were not tested for glucose tolerance were euthanased in the same way. In these animals the pancreas, liver and kidney were fixed overnight in 10% buffered formalin for histology and immunocytochemistry. The liver, kidney, muscle and fat were also frozen in these animals for the determination of lipid deposits.

2.11 Statistical Analysis

The data was analysed using the Graph Pad Prism package. Values are reported as mean \pm SEM. The variance between the groups was done using ANOVA. The line graphs were plotted on Microsoft Excel and the bar charts were done on Prism.

CHAPTER 3

Results

3.1 Weight

The changes in the body weight of the various groups of rats over the period of study are shown in figures (3.1a-3.1g). There was no significant difference in MD fed animals before treatment when compared to the control (Fig. 3.1g). Even during the treatment period they did not show a significant difference to the control group. However significant difference was shown by the animals that were fed HFD when their weights were compared to that of the control group before treatment (Fig3.1a-e). The rats treated with C2, metformin and rosiglitazone showed significant difference in weights when compared with the control group (Fig 3.1b). Other groups on HFD were also heavier than control but their weight was not significantly different. After one week of treatment more groups were significantly heavier than the control group. After two weeks of treatment the groups that were fed HFD showed significantly higher weight than control except for the group that was treated on C5. From week three to the completion of the treatment period the untreated, metformin and rosiglitazone treated groups showed significantly different weight compared to the control group. Except for week two and nine of treatment whereby the group treated with C4 was significantly different from the control group, C4 and C5 showed no significant difference to the control (Fig 3.1d and e). The groups treated with C2 and C3 were significantly different to the control except during the last week of treatment (Fig 3.1b-c). The group treated with C1 showed significant difference in some weeks of treatment and no significant difference in other weeks (Fig 3.1a).

When treatment groups for the group that was fed HFD were compared to each other significant difference was only seen in some but not all the groups (Fig 3.1g). The groups that were treated with C1 and C4 showed significant difference during week nine, ten and eleven when compared to the metformin treated group. During week twelve the C1 treated group showed significant difference to the metformin and the untreated group. C1 treated group started to show significant difference during the sixth week of treatment, but before that there was no significant difference amongst the groups.

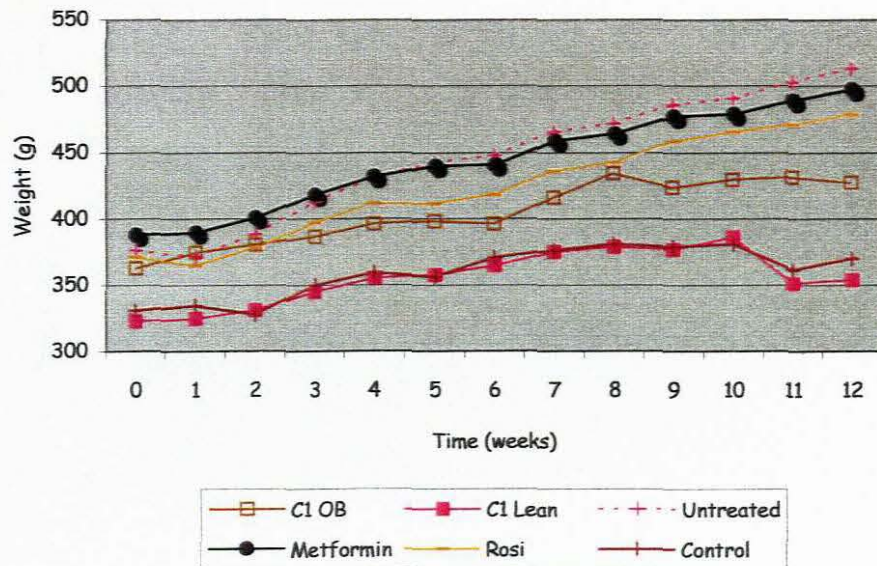


Figure3.1a Weights for the groups treated with C1 for MD and HFD fed groups compared to control, untreated and other treatment groups

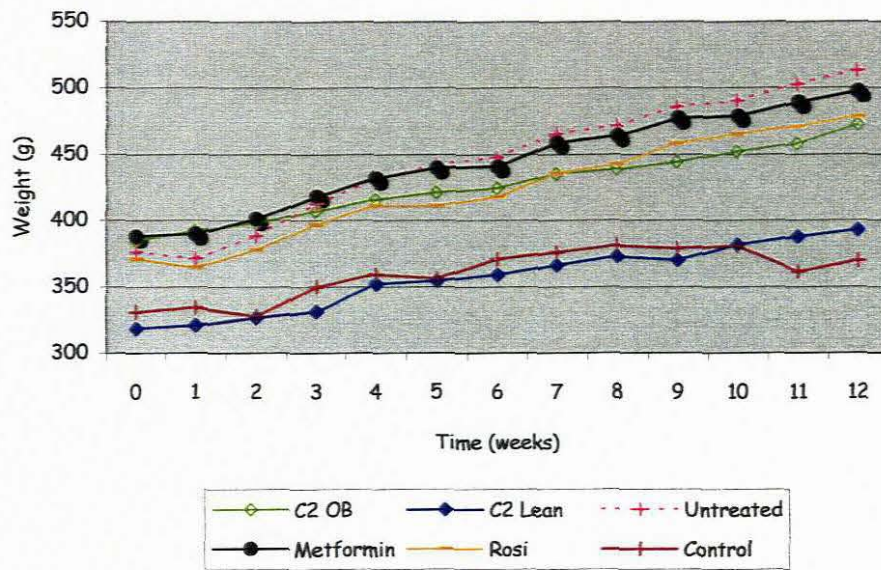


Figure 3.1b: Difference between HFD and MD fed animals treated with C2

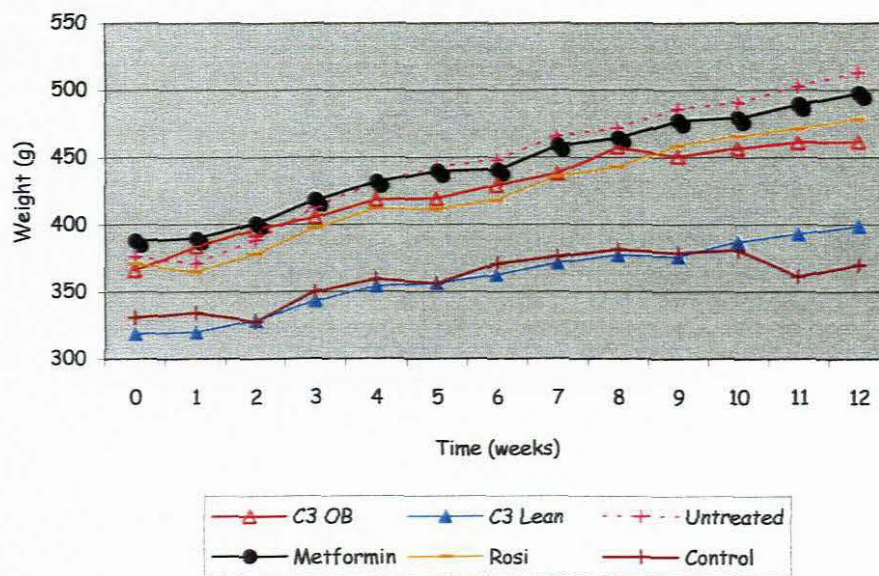


Figure 3.1c: Weight of animals treated with C3 and fed either HFD or MD

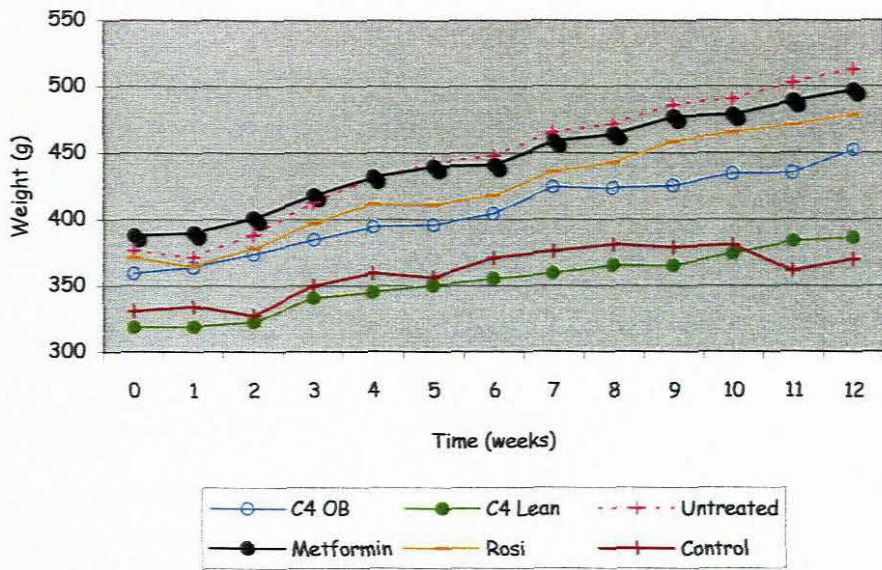


Figure 3.1d: Differences in weights of animals treated with extract C4, fed MD or HFD

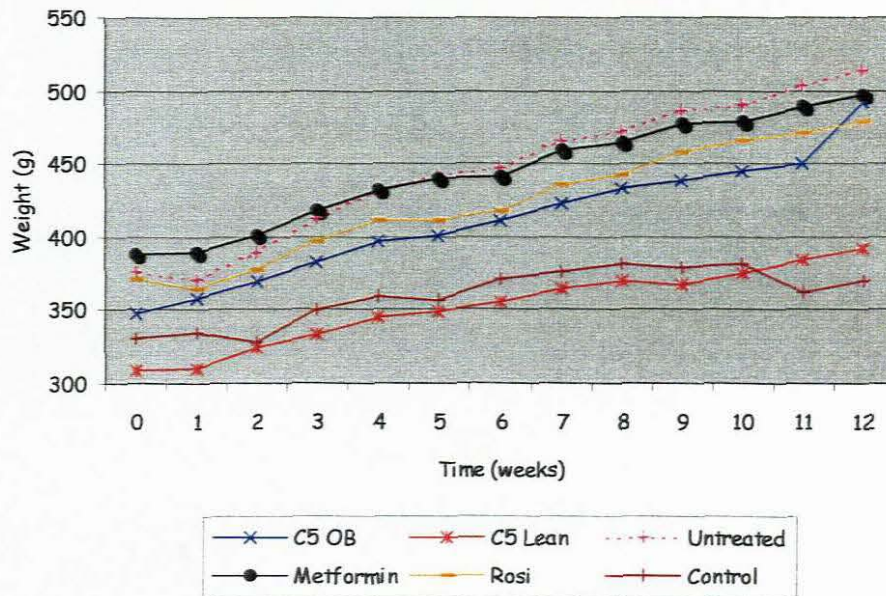


Figure 3.1e: Body weights of animals treated with C5 compared with other treatments for animals treated with HFD or MD

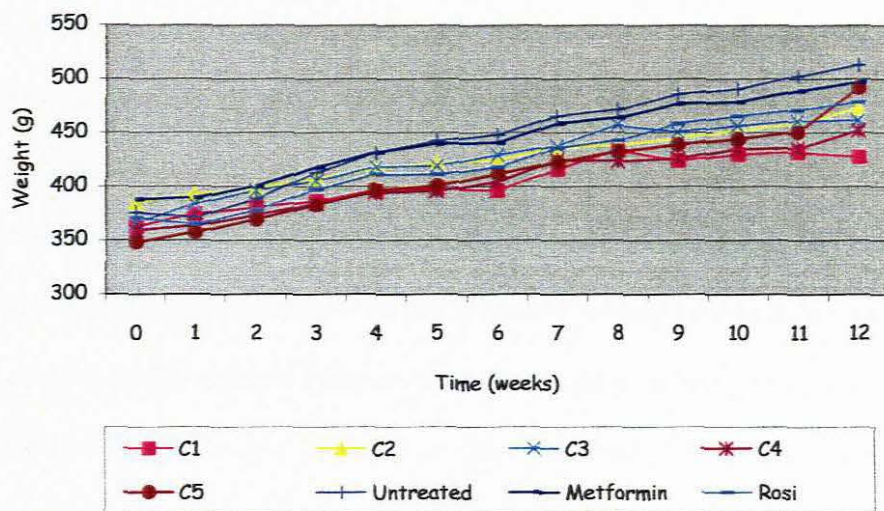


Figure 3.1f: Weight gain for animals fed HFD compared to each other from baseline throughout the experimental period

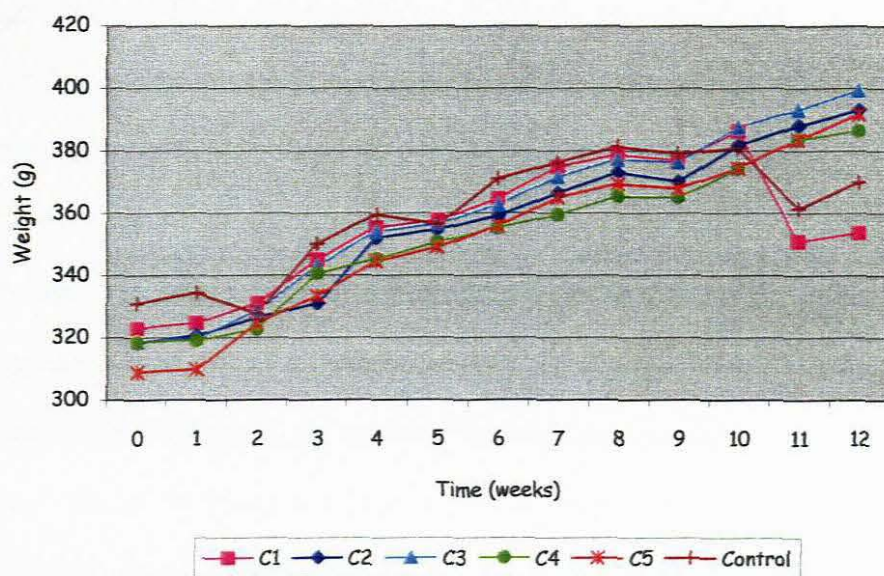


Figure 3.1g: MD fed rats' weights through experimental period

3.2 Food intake

The amount of food intake differed for the groups when compared (Fig 3.2a-g). After one week of treatment the groups that were fed MD and treated with C3 and C4 showed a significantly different intake of food compared to the control (Fig 3.2f). No significant difference was seen after two weeks of treatment for the treated groups compared with the control. Significant difference was seen on the third week of treatment with groups treated with C1, C2 and C3 having lower food intake compared to control. There were no differences in food intake for the groups fed MD between week four and week eight of treatment. The difference in food consumption also took place during the ninth week of treatment with C1, C2 and C3 also showing significant difference to the control group (Fig 3.1a-c). Week ten through the end of the treatment period there was no significant difference seen.

Food intake differed significantly for the HFD fed groups when compared to the control (Fig 3.2a-e). After one week of treatment groups that were treated with an extract had significantly lower food intake than the control. There was no significant difference for the groups that were treated with metformin and rosiglitazone, the untreated group also showed no significant difference after the first week of treatment. After the second week of treatment there was no significant difference for all the groups when compared to the control. The metformin treated group and the untreated group showed significantly higher and lower food intake respectively, in the third week of treatment. Other groups had no significant difference. In the fourth week it was the rosiglitazone and metformin treated groups that showed significantly higher food intake to that of the control. During week five of treatment the group treated with metformin and the untreated group showed significantly higher and lower food intake

respectively, with the rest of the treatment groups showing no significant difference. From the sixth week the groups treated with metformin and rosiglitazone showed significantly higher food intake compared to the untreated group and this was seen up to the last week of treatment. The extract treated groups showed significant difference in food intake in some of the weeks when compared to the control group. The groups that were fed C3 and C4 had significantly different food intakes to that of the control group from week six to week twelve of treatment, excluding week seven, nine and ten. The groups that were treated with C1 and C2 showed a significant difference between week six and twelve of the treatment period, with no significant difference during week six, nine and ten. The group that was treated with C5 had no significantly different food intake when compared to the control group between week six and twelve, with significant difference only during weeks eight and twelve (Fig 3.2e).

The HFD fed groups showed no significant difference in food intake after the first week of treatment. The untreated group, the metformin and rosiglitazone treated groups had significantly high food intakes compared to extract treated groups. During the second week of treatment only the group treated with C3 showed a significant difference compared to rosiglitazone and metformin (Fig 3.2g). In the third week the rosiglitazone treated group had no significant difference when compared to the extract treated groups. The untreated group had significantly lower food intake than the treated groups. The food intake by the metformin treated group was significantly higher than that of the rosiglitazone and extract treated groups in the third week of treatment. During the fourth week of treatment the extract treated groups were significantly different in food intake compared to the metformin and rosiglitazone treated groups. The untreated group was not significantly different compared to

extract treated animals except for the group that was treated with C2. The intake of the metformin treated group was significantly different to that of the rosiglitazone treated group. Except for week five the food intake for the rosiglitazone and metformin treated groups was not significantly different after four weeks of treatment to the end of the treatment period. The food intake for the treated groups showed significant difference after five weeks of treatment to the end of treatment except for weeks six and ten. The group that was treated with C3 showed significant difference in food intake to the rosiglitazone treated group from week five to the end of the treatment period, except for week eleven and twelve. The rosiglitazone treated group had no significantly different food intake between weeks five and twelve compared to the C5 treated group. The difference was significant only for weeks six, nine and ten. The metformin group showed significantly higher food intake than the extract treated animals except during the last week of treatment.

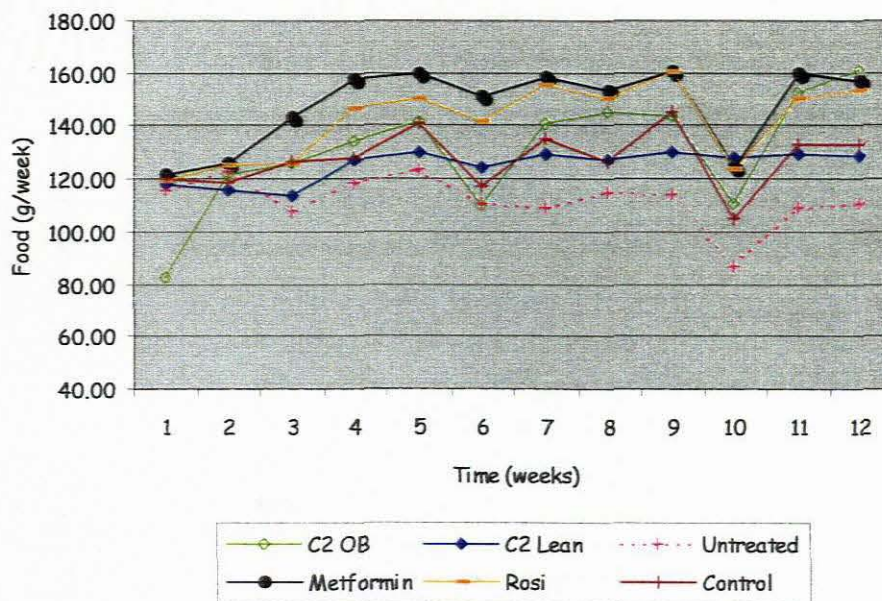


Figure 3.2a: Comparison between C1 treated animals' food intake to other treated and untreated animals

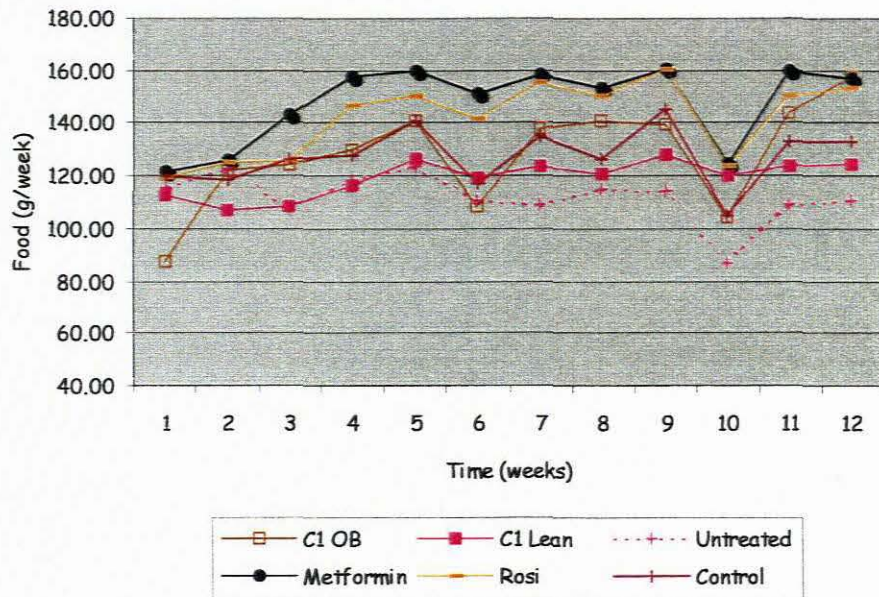


Figure 3.2b: Food consumption for C2 fed animals compared with drug treated, untreated and control

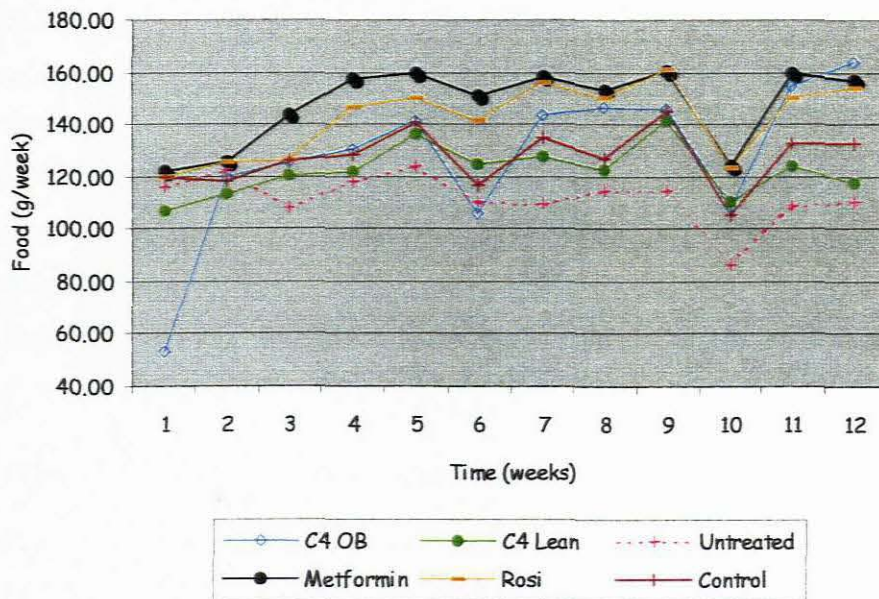


Figure 3.2c: The effect of C3 in the food intake of HFD and MD fed animals in comparison with other untreated, drug treated and control

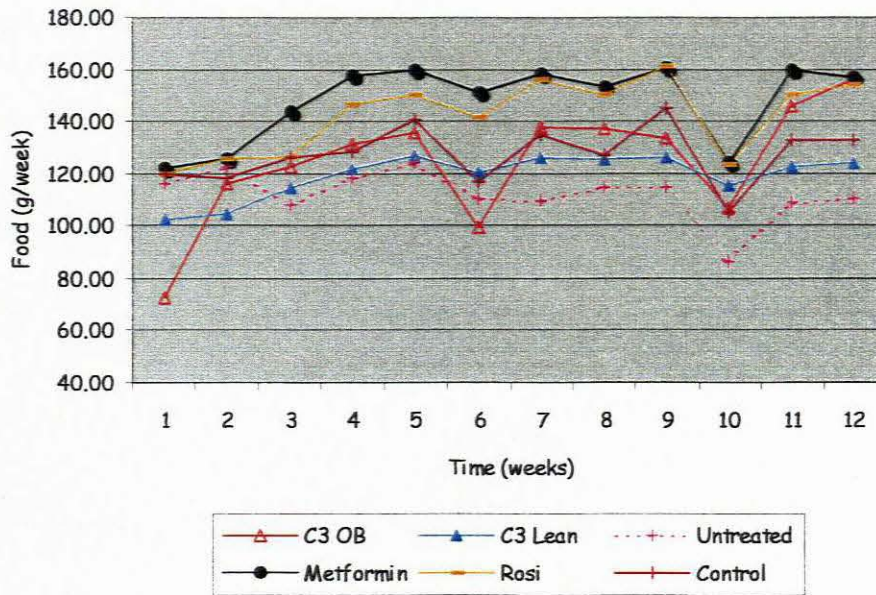


Figure 3.2d: The effect of C4 in food intake of HFD and MD fed animals

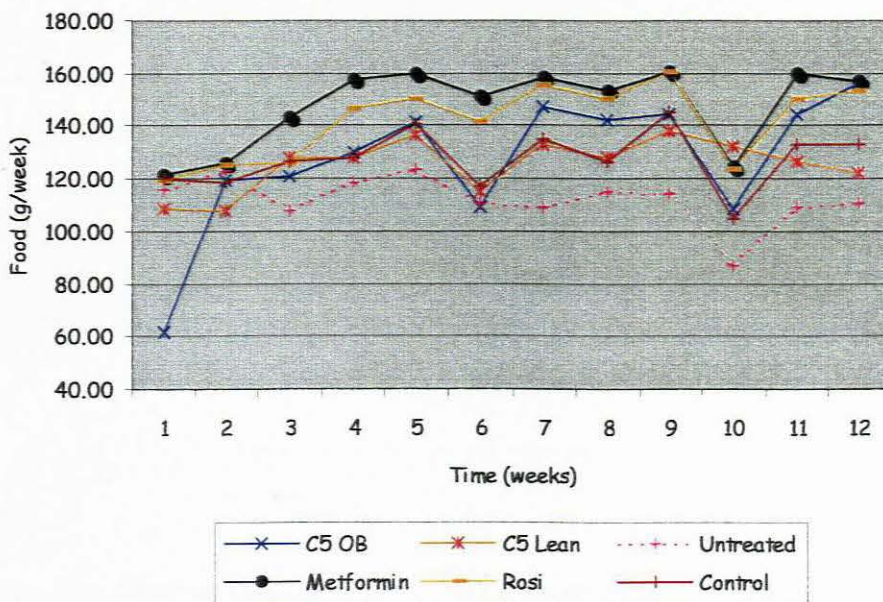


Figure 3.2e: Food intake for C5 treated groups on MD or HFD compared to other treatment groups

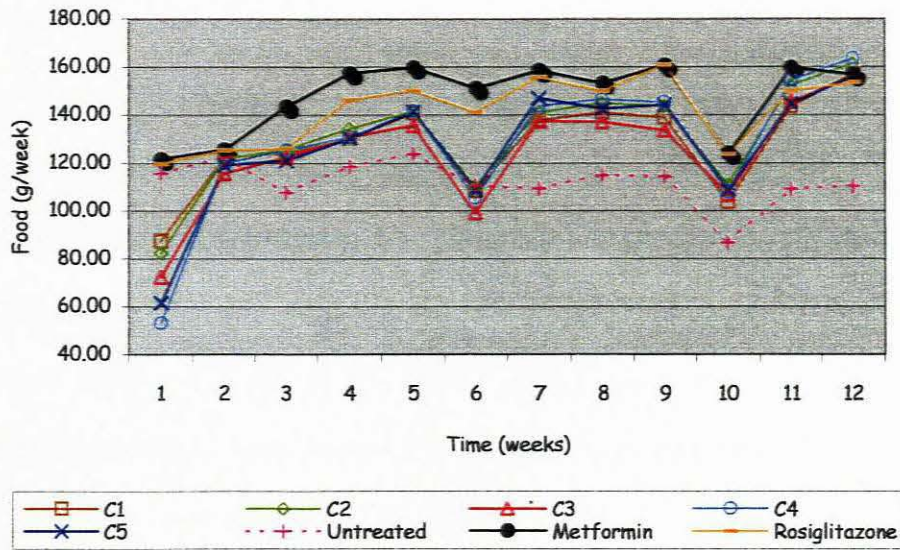


Figure 3.2f: Food intake within the obese model for all the treatment groups

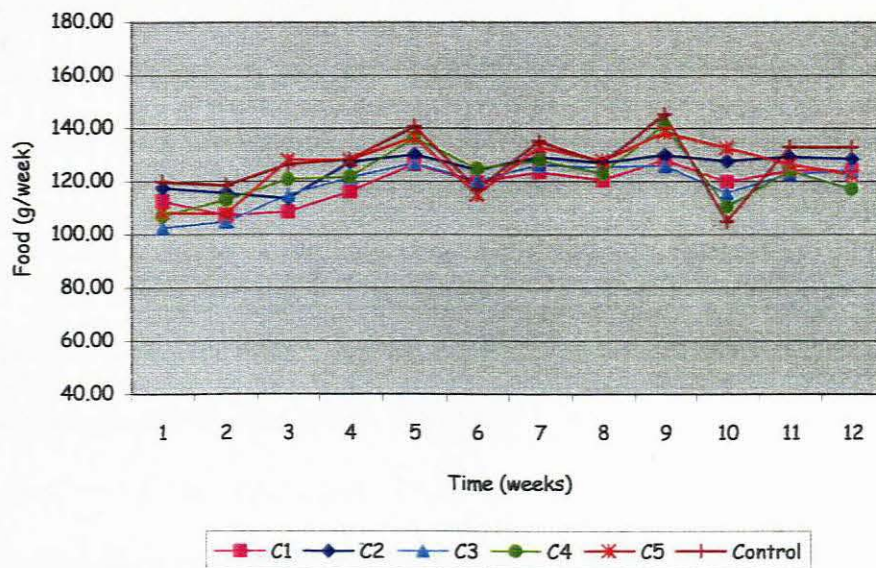


Figure 3.2g: Differences in food intake for groups treated with different extract concentrations and fed MD in comparison with the control group.

3.3 Blood glucose

The level of blood glucose in the various rat groups are presented in figures (3.3a-g). The MD fed groups had no significant difference in blood glucose level compared to the control group before treatment. The difference was also not significant during the treatment period (Fig.3.3f). Although the MD fed groups did not show a significant difference when compared to the control group, some HFD fed groups were significantly different to the control group in blood glucose level before treatment (Fig 3.3a). The untreated group, rosiglitazone, metformin and C1 treated groups showed significantly high blood glucose levels. The extract treated groups that were treated with C2-C5 were not significantly different to the control group (Fig 3.3b-d). The group that was treated with C3 showed significantly low blood glucose after four weeks treatment, with the rest of the treatment groups that were fed HFD, having no significant difference to the control (Fig 3.3c). The glucose levels for the HFD fed group had no significant difference to that of the control after weeks eight and twelve (Fig 3.3a-e).

When comparing the groups that were fed HFD to one another a significant difference was not seen before treatment. After four weeks of treatment the group that was treated on C3 was significantly lower in blood glucose level compared with the untreated, rosiglitazone and metformin treated groups. The group treated with C5 also showed a significant difference to the rosiglitazone treated group after eight weeks of treatment. The difference in blood glucose level was not significantly different for all the groups after twelve weeks of treatment (Fig 3.3g).

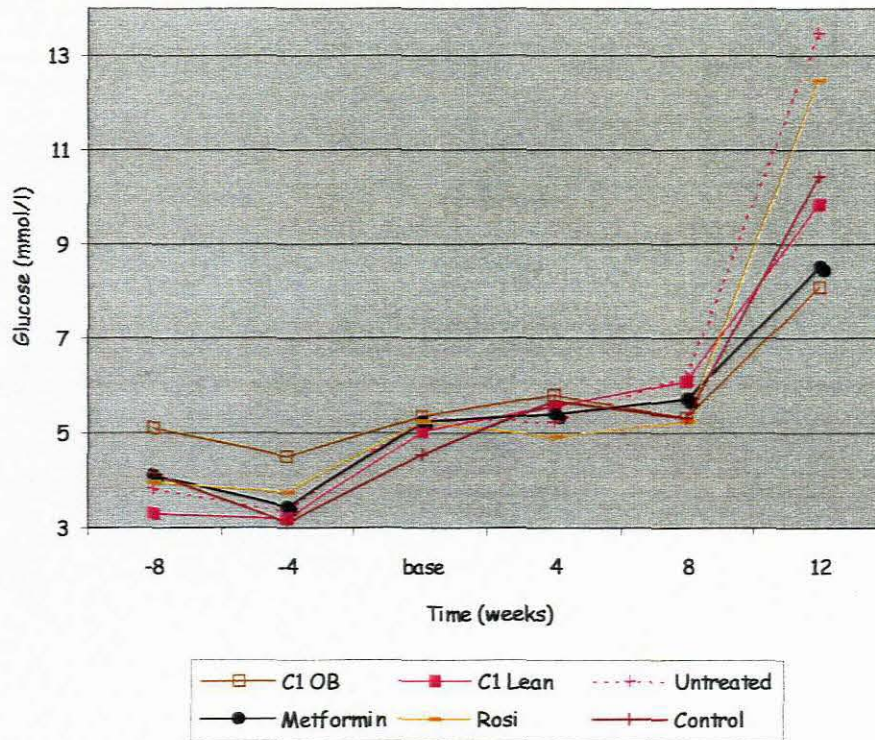


Figure 3.3a: Glucose levels of animals treated with C1 compared with the untreated group, control and animals treated with commercial drugs

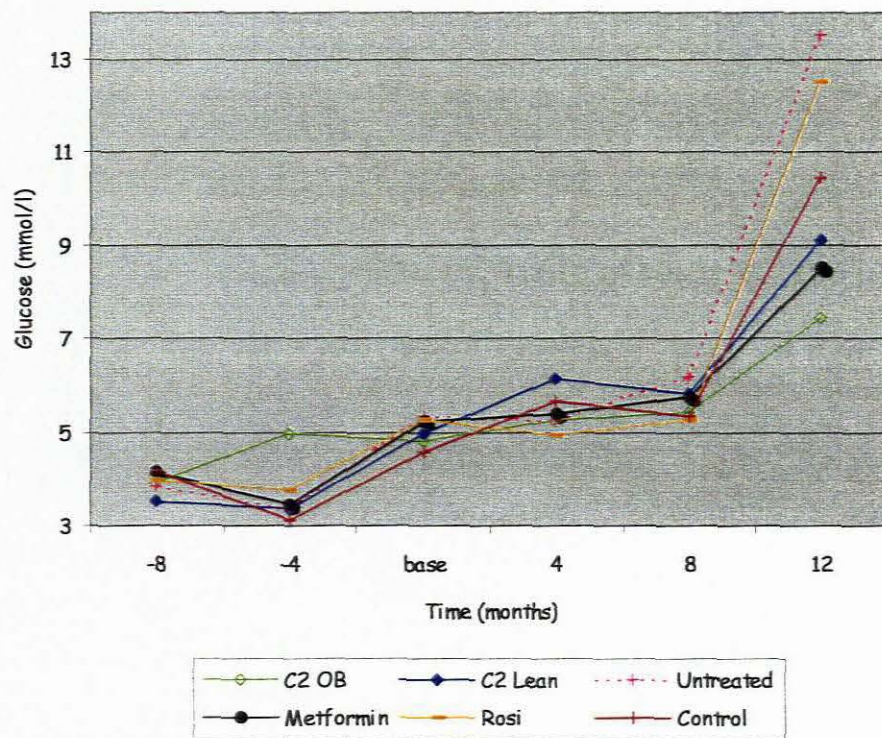


Figure 3.3b Glucose levels of animals treated with C2 compared with the untreated group, control and animals treated with commercial drugs

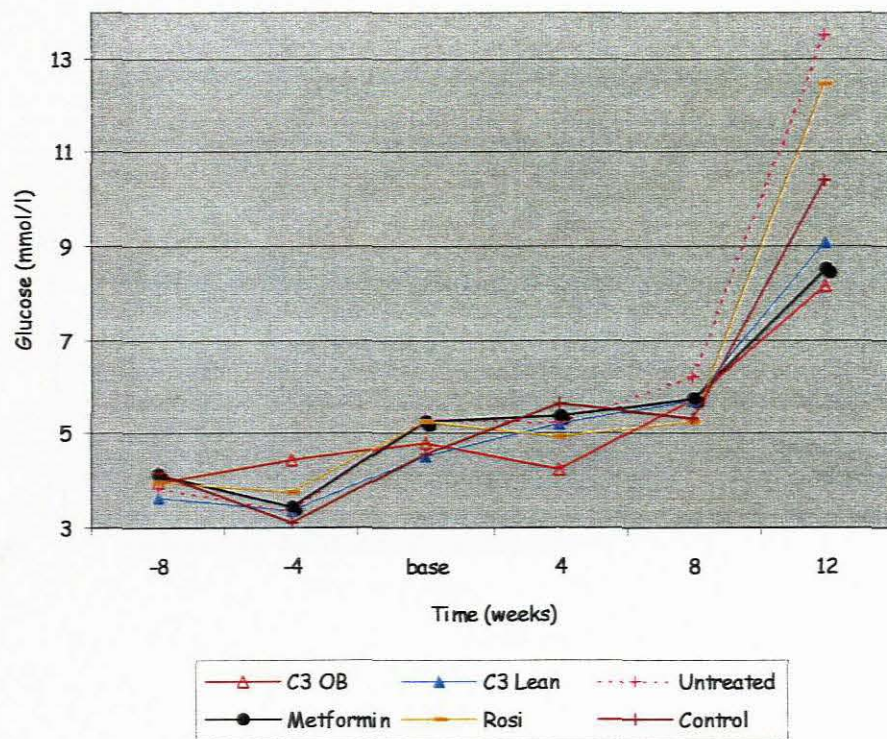


Figure 3.3c: Glucose levels of animals treated with C3 compared with the untreated group, control and animals treated with commercial drugs

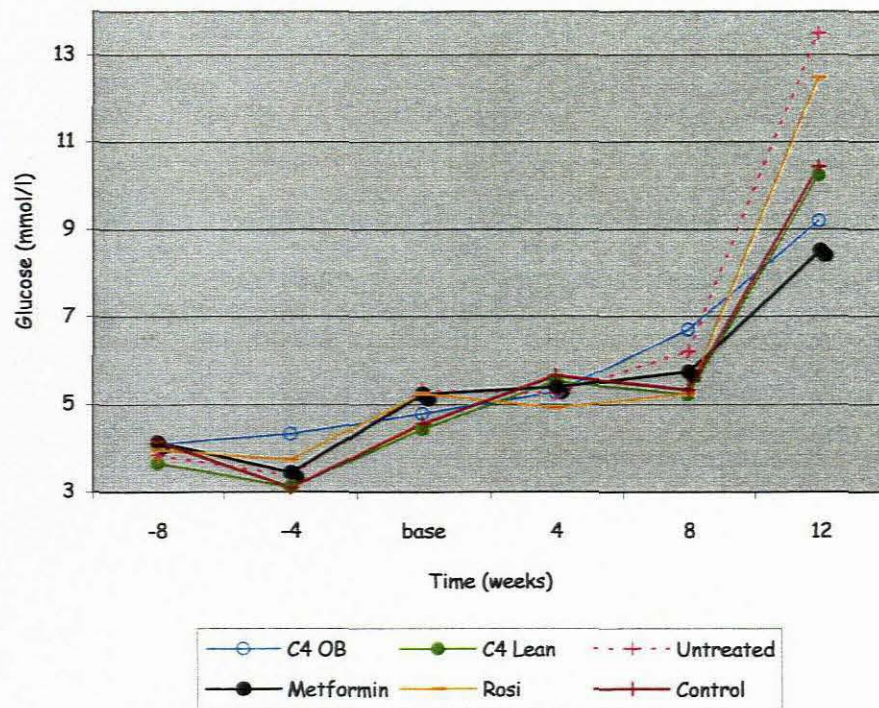


Figure 3.3d: Glucose levels of animals treated with C4 compared with the untreated group, control and animals treated with commercial drugs

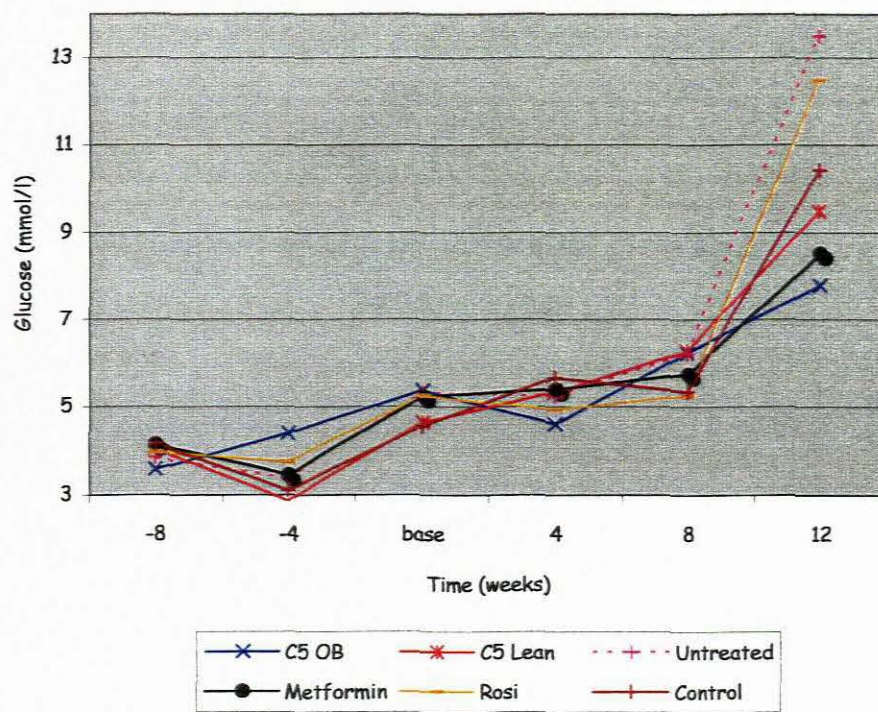


Figure 3.3e: Glucose levels of animals treated with C5 compared with the untreated group, control and animals treated with commercial drugs

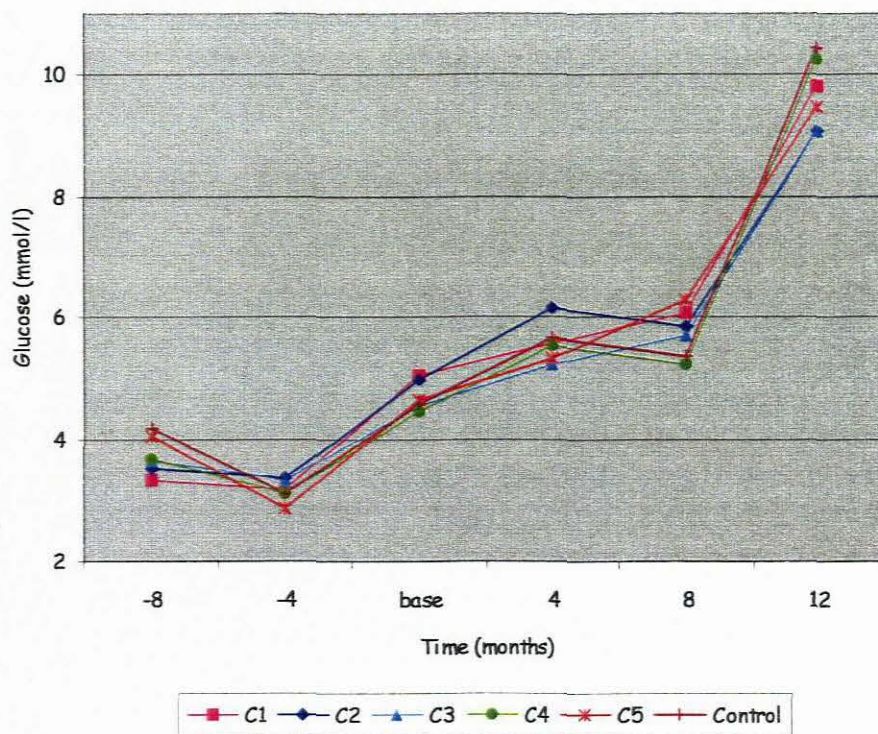


Figure 3.3f: Glucose levels for the animals that were fed MD before and after treatment

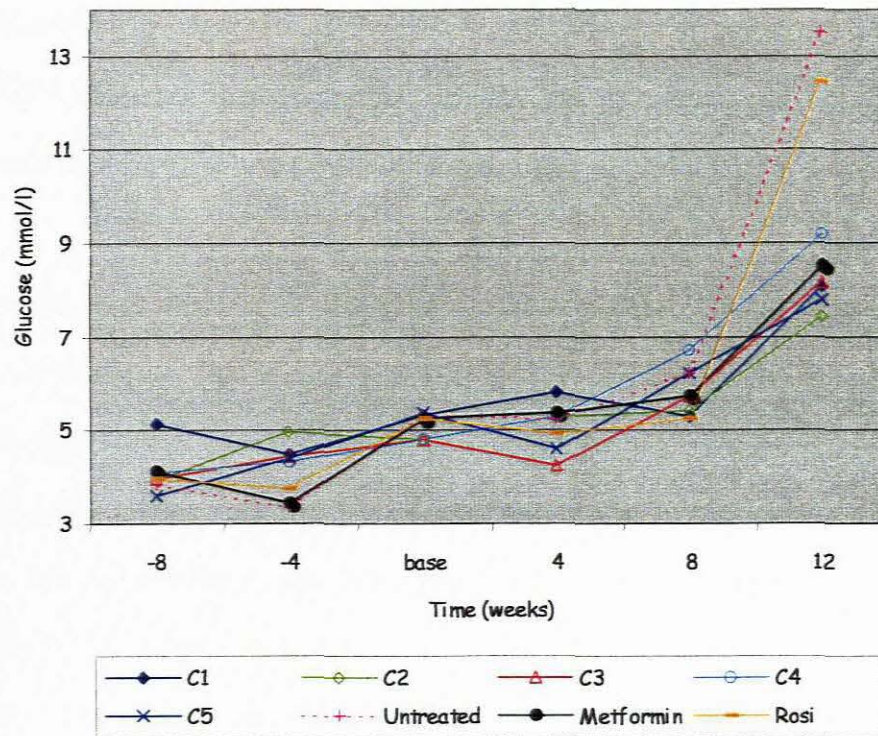


Figure 3.3g: HFD fed groups' glucose levels before and after treatment

3.4 Insulin levels:

Figures 3.4a-3.4g show the measured insulin levels of the rats in the various groups. The baseline values of insulin were not significantly different for the group fed the MD diet before treatment. After four weeks of treatment the groups that were treated

with C2 and C3 had significantly higher and lower insulin levels when compared to the control group respectively. The rest of the groups did not show significant difference to the control group when compared after four weeks of treatment. After eight weeks treatment the insulin level of the C5 treated group that was fed a MD rose significantly higher compared to the control, with the rest of the groups having no significant difference to the control. The MD fed groups showed no significant difference in insulin levels to the control group after twelve weeks (Fig 3.4f).

The insulin levels for the HFD fed groups were significantly high before treatment when compared to the control group (Fig 3.4a-e). The levels were still not significantly different after four weeks of treatment for the HFD fed groups, except for the group treated with rosiglitazone. After eight weeks of treatment only the groups that were treated with the extract had significantly high insulin levels compared to the control group (Fig 3.4a-e). The level of insulin for the groups that were fed HFD was not significantly different to the control group when they were compared after twelve weeks.

Animals that were fed HFD did not show significantly different insulin levels before the start of the treatment (Fig 3.4g). The rosiglitazone group, which had low insulin levels after four weeks of treatment, was significantly different to the extract treated groups except the C5 and untreated group. The metformin and untreated groups had low insulin levels but they were not significantly different to the extract treated groups. The group that was treated with C4 had significantly higher insulin levels compared to rosiglitazone and metformin treated groups. The low levels of the rosiglitazone treated group were significant, also when compared to the C1 and C3

treated groups after eight weeks (Fig 3.4a and c). After twelve weeks the insulin levels for the untreated group were significantly high compared to the metformin and C1 treated group, with no significant difference compared to other treatment groups (Fig 3.4a).

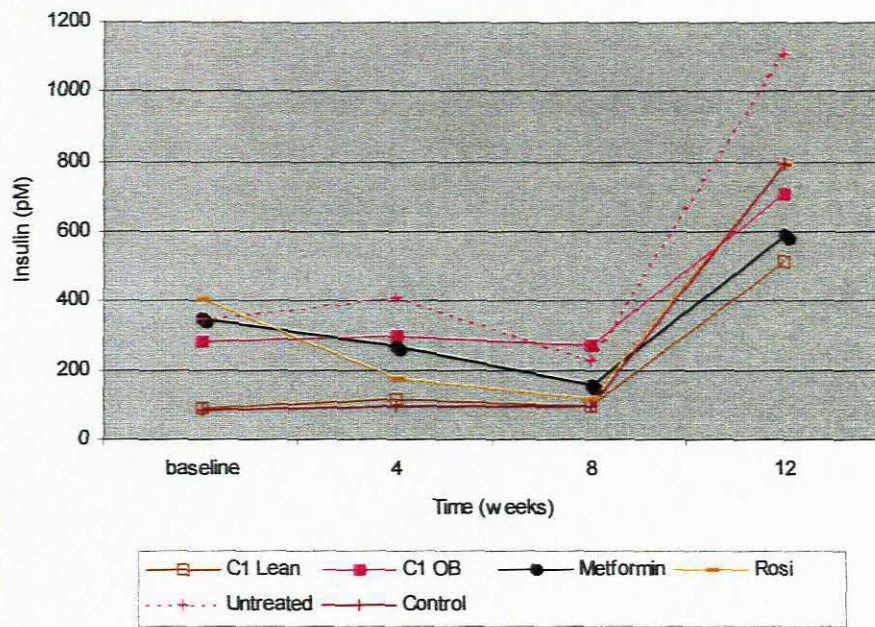


Figure 3.4a: Insulin levels of C1 treated animals on either MD or HFD compared to that of the control; untreated or drug treated animals

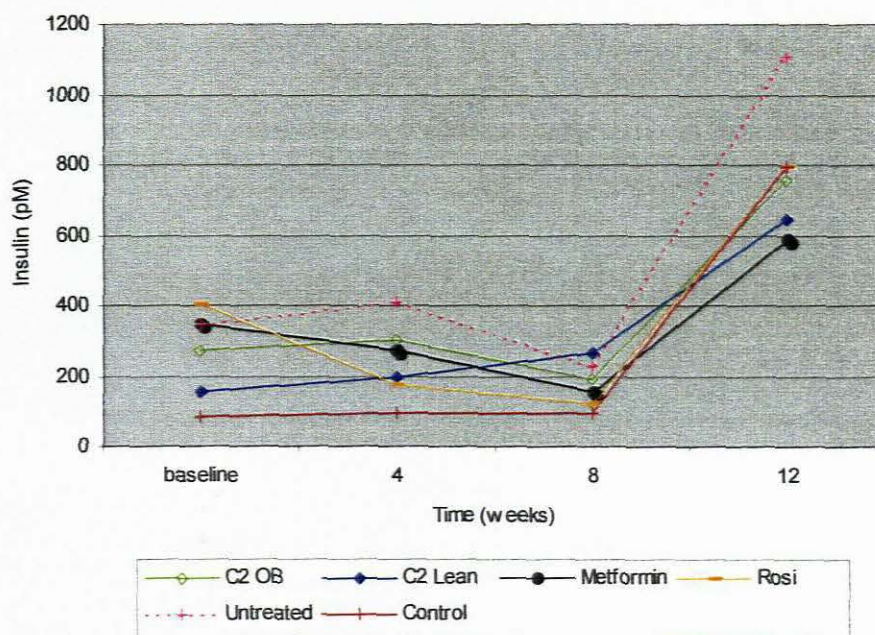


Figure 3.4b: Insulin levels of C2 treated animals

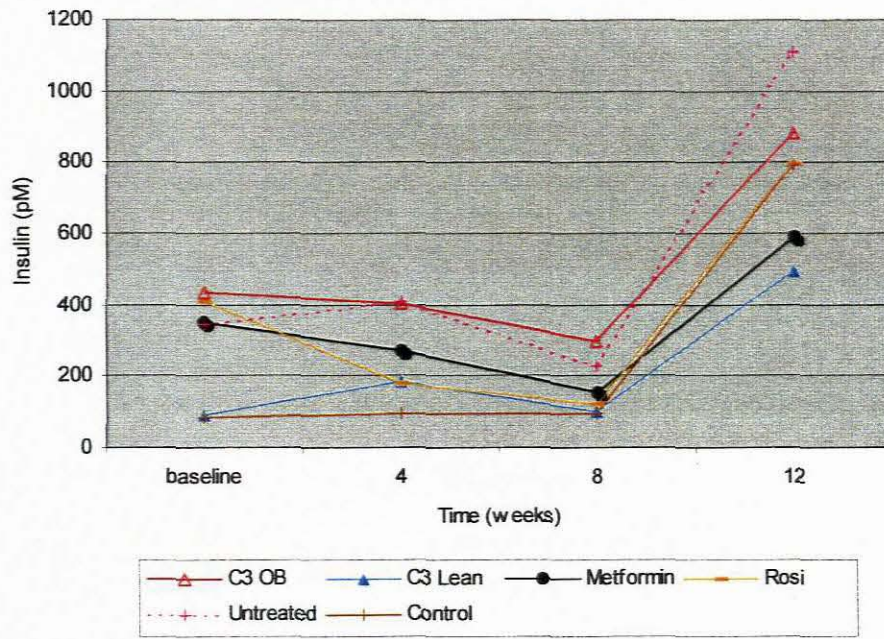


Figure 3.4c: The effect of C3 in insulin levels of MD or HFD fed animals and other treatment groups

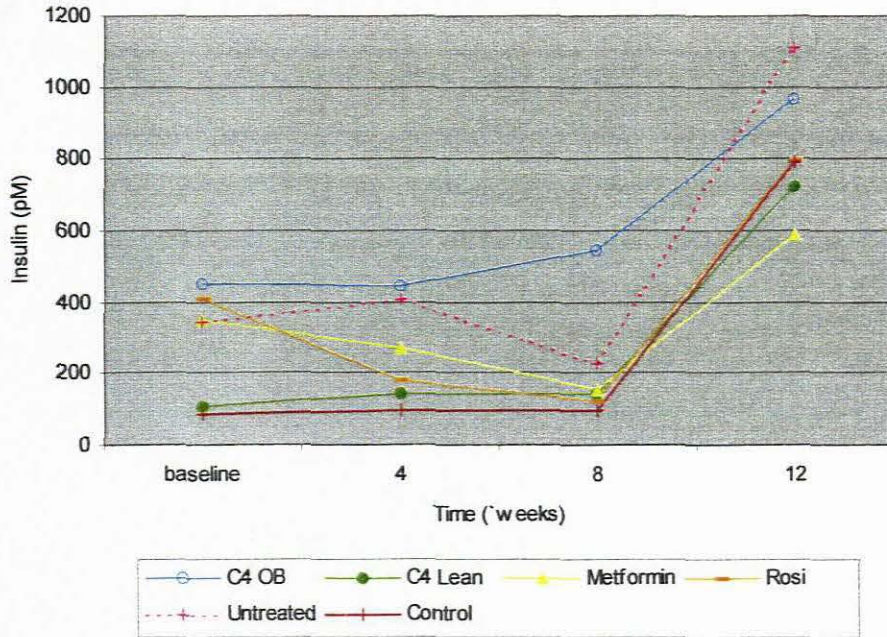


Figure 3.4d: Insulin levels of C4 treated groups

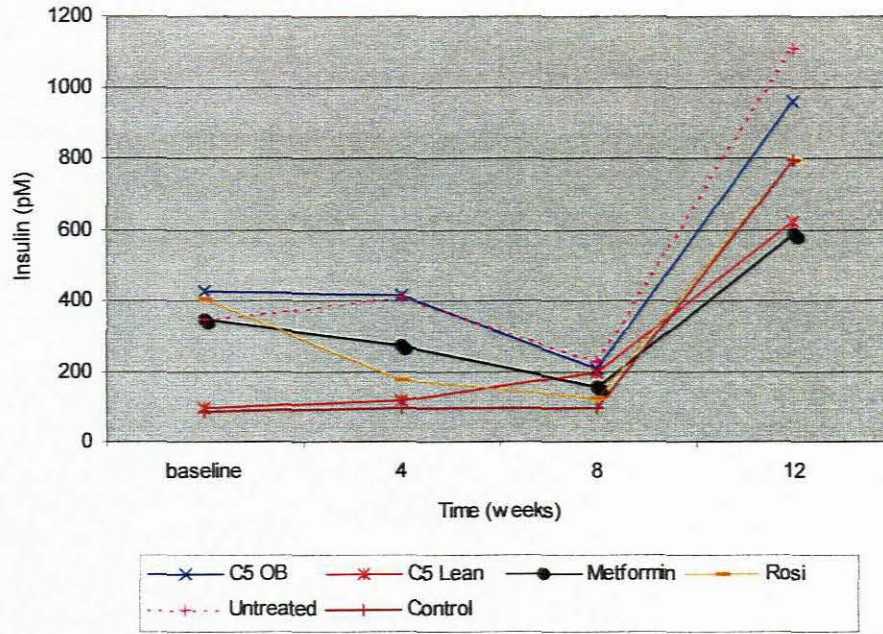


Figure 3.4e: C5 insulin levels compared to untreated, control and drug treated animals

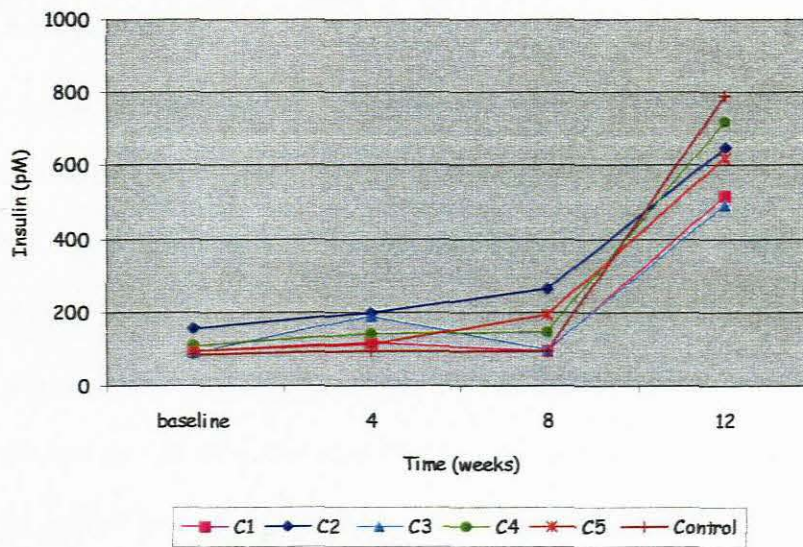


Figure 3.4f: Insulin levels for the MD fed groups from baseline to the end of treatment period

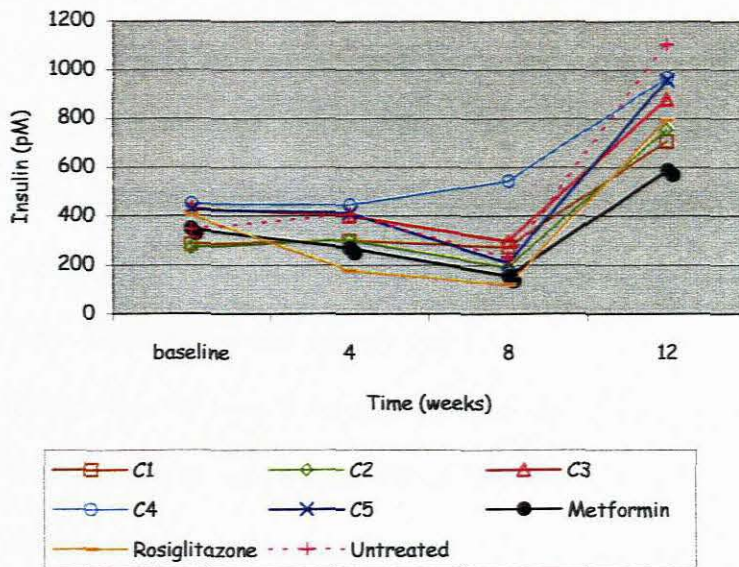


Figure 3.4g: Insulin levels for the MD fed groups from baseline to the end of treatment period

3.5 Lipid levels

Cholesterol levels for the groups that were fed MD were not significantly different to that of the control group (Fig 3.5a). But the groups that were treated with C1 and C5 had significantly low levels of total cholesterol. The levels of TAG and HDL cholesterol levels were not significantly different to that of the control for MD fed groups treated with extract (Fig 3.5b and c). The group that was treated with C5 showed significantly low LDL levels compared to the control group, with the rest of the groups showing no significant difference to the control (Fig 3.5d).

A significant difference was seen for the HFD fed groups when compared to the control group. The total cholesterol levels for the groups treated with C3, C4 and C5 were significantly low compared to that of the control group (Fig 3.5e). Other groups

that were fed HFD had no significantly different total cholesterol levels. Total cholesterol level for the rosiglitazone treated group differed significantly from the control (Fig 3.5e). Except for the C5 treated group the groups that were treated with extract showed significantly different TAG levels compared to the control group (Fig 3.5f). The untreated group was significantly different in TAG level to the control group. The metformin and rosiglitazone treated groups had no significantly different TAG levels to the control group (Fig 3.5f).

The groups that were treated with extract showed significantly low HDL cholesterol levels compared with the control group. The HDL level of the untreated group was not significantly different to that of the control group and similar levels of HDL were shown by the metformin and rosiglitazone treated groups (Fig 3.5g).

The levels of LDL cholesterol were significantly low compared to that of the control group. The groups that were treated with metformin and extract showed no significant difference to the control for levels of LDL. The untreated group showed no significant difference in LDL, also when compared with the control group (Fig3.5h).

When comparing the HFD fed groups in different treatments the difference was seen in the total cholesterol being significantly high for the metformin treated group than for any other group (Fig 3.5e). The rosiglitazone treated group showed no significant difference in total cholesterol levels compared with both the untreated and extract treated groups (Fig. 3.5e). TAG levels were high for the group treated with C4 and the untreated group, leading to significantly different levels when compared to the metformin and rosiglitazone treated groups, but with no significant difference when they were compared to each other (Fig 3.5f). The group that was treated with C1 showed significantly high TAG levels when compared to the rosiglitazone group. The

rest of the extract treated groups did not show any difference when compared with the metformin and rosiglitazone groups. The untreated group showed significantly high TAG levels, also when compared with the C5 treated group.

Significantly high HDL levels for the metformin treated group compared to extract treated groups were seen after treatment. The rosiglitazone treated group also showed high HDL levels for the groups that were treated with C1-C3 and no significant difference compared to C4 and C5. The untreated group also had significantly high levels of HDL, compared to extract treated animals given C1-C3. There was no significant difference between the untreated, rosiglitazone and metformin treated groups (Fig 3.5g). The untreated group showed no significantly different LDL levels to any treated group. The rosiglitazone and metformin groups showed significantly different LDL levels with the metformin group having high levels of LDL. Significantly low LDL levels were seen for the rosiglitazone treated group when compared to C1 and C2 treated groups which had high LDL levels. When the metformin group was compared with extract treated animals the groups that were treated with C4 and C5 had significantly low LDL levels (Fig 3.5h).

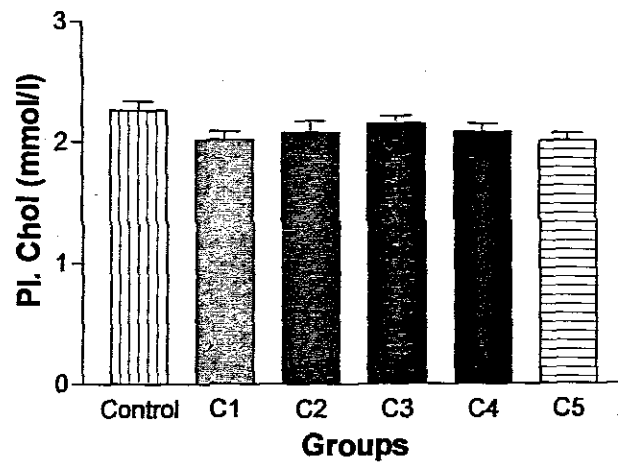


Figure 3.5a: Plasma total cholesterol levels of extract treated and MD fed groups compared to that of a control

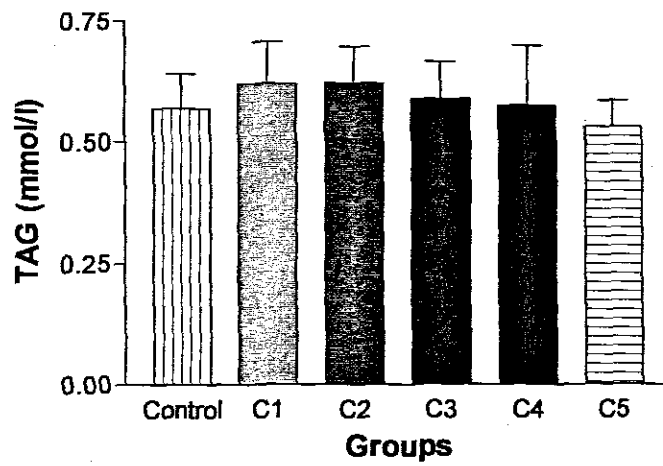


Figure 3.5b: Plasma total cholesterol levels of extract treated and MD fed groups compared to that of a control

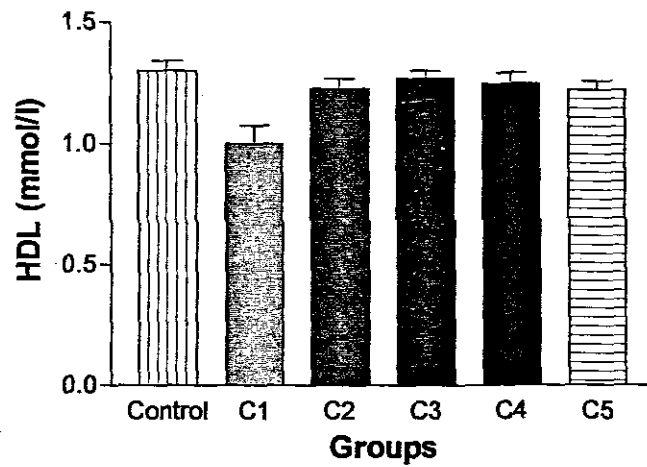


Figure 3.5c: HDL cholesterol levels of extract treated and MD fed groups compared to that of a control

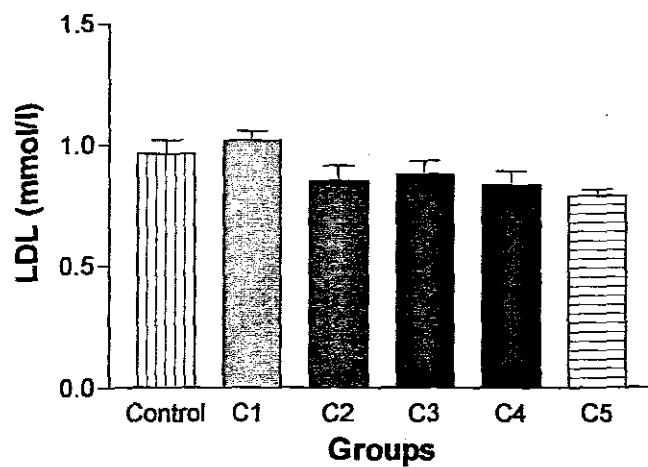


Figure 3.5d: LDL cholesterol levels of extract treated and MD fed groups compared to that of a control

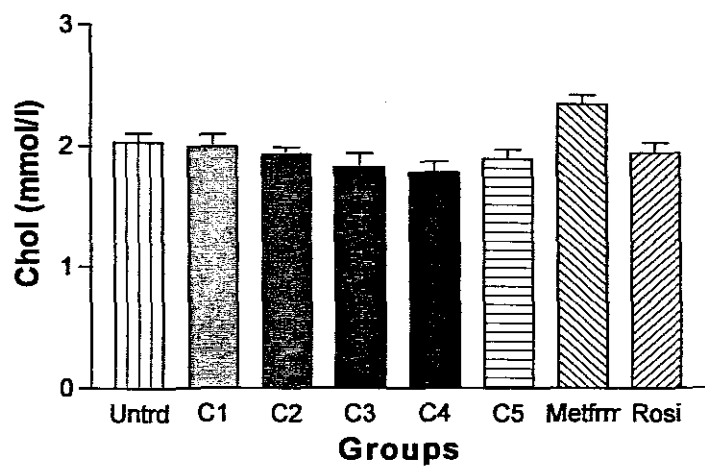


Figure 3.5e: Total plasma cholesterol levels of extract treated and HFD fed groups compared to that of other groups

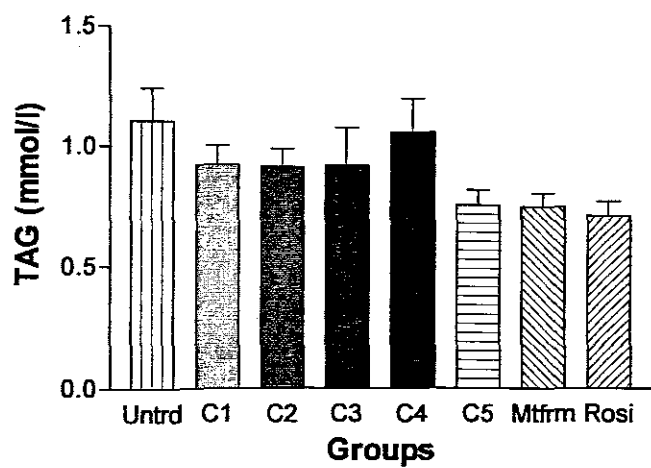


Figure 3.5f: TAG cholesterol levels of extract treated and HFD fed groups compared to that of other groups

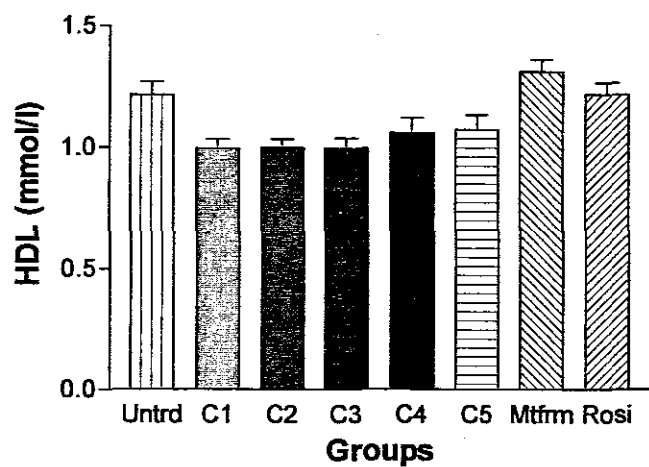


Figure 3.5g: HDL cholesterol levels of extract treated and HFD fed groups compared to that of a control

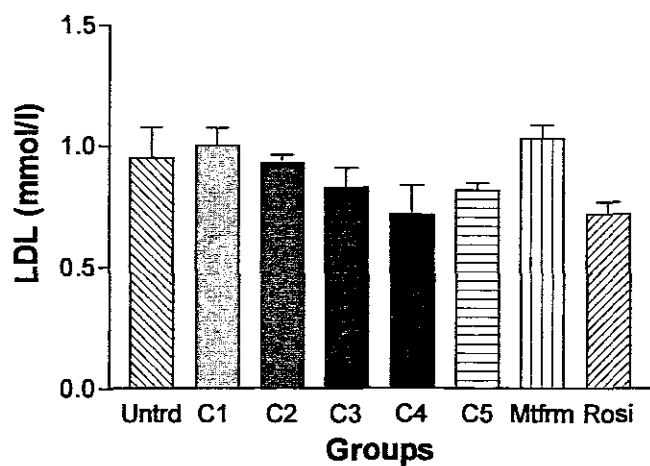


Figure 3.5h: LDL cholesterol levels of extract treated and HFD fed groups compared to that of a control

3.6 Glucose clearance:

Values from the IVGTT were used to determine the area under the curve for each animal in a group and values found were used to calculate the glucose clearance per group. There was no significant difference in glucose clearance for both groups on MD or on HFD when compared to the control group (Fig 3.6a and b). High glucose clearance was seen for the groups treated with extract compared to the control group for the groups on MD (Fig 3.6a). The group treated with C1 showed it to be the only group to have glucose clearance lower than the control group for the groups on MD. The groups treated with C2 and C4 showed the highest glucose clearance respectively. The groups on HFD showed different glucose clearance to that of the MD fed groups. There was a poor glucose clearance for the groups treated with C2 and C4 compared to the control group (Fig 3.6a). The group treated with C5 showed the highest glucose clearance. The groups treated with metformin and rosiglitazone also showed higher glucose clearance compared to the control group. There was higher glucose clearance for the untreated group in comparison with the control group and some of the extract treated groups.

The group treated with C5 showed the highest glucose clearance than all the HFD fed groups. The group treated with metformin had a higher glucose clearance rate than the extract treated, rosiglitazone treated and untreated groups. The untreated group showed higher glucose clearance than groups treated with C2 and C4. The

rosiglitazone treated group showed a higher glucose clearance level compared to extract treated groups except C5 and untreated group (Fig 3.6b).

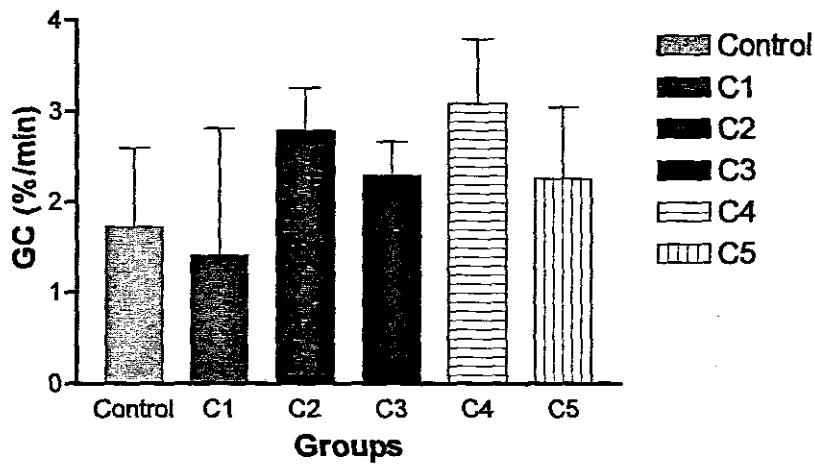


Figure 3.6a: Glucose clearance of animals treated with extract and fed MD

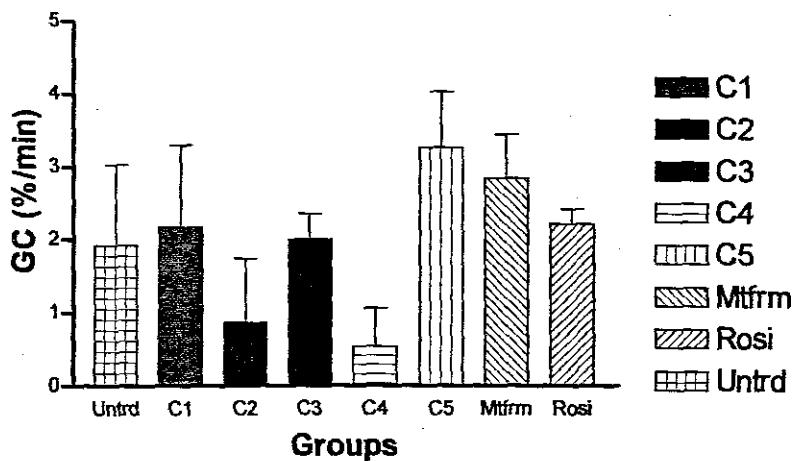


Figure 3.6b: Glucose clearance of animals treated with extract and fed HFD

3.7 Effect of dosage to weight for groups treated with extract

The dosage of an extract for the groups was different. The groups on C1 extract showed to have a low dose of an extract compared with groups on other concentrations (Fig 3.7f and g). This was seen for both the groups on HFD and MD. There was a difference in dosage between the groups on HFD when compared with the groups on MD. For all the extract concentrations C1 to C5 the groups on MD had a high dosage (Fig 3.7a-e). There was a correlation between the dosage and percentage weight gain for all the groups. The increase in dosage caused the increase in the percentage weight gain. These results showed the relationship between the dosage, weight gain and concentration. The increase in concentration was the increase in dosage and hence the increase in body weight gained.

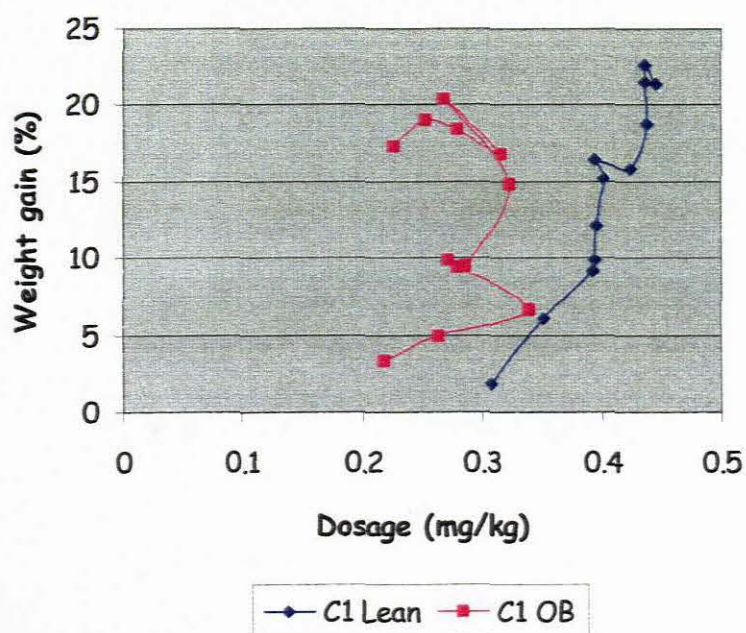


Figure 3.7a: The effect of C1 dosage in weight for animals fed HFD and MD

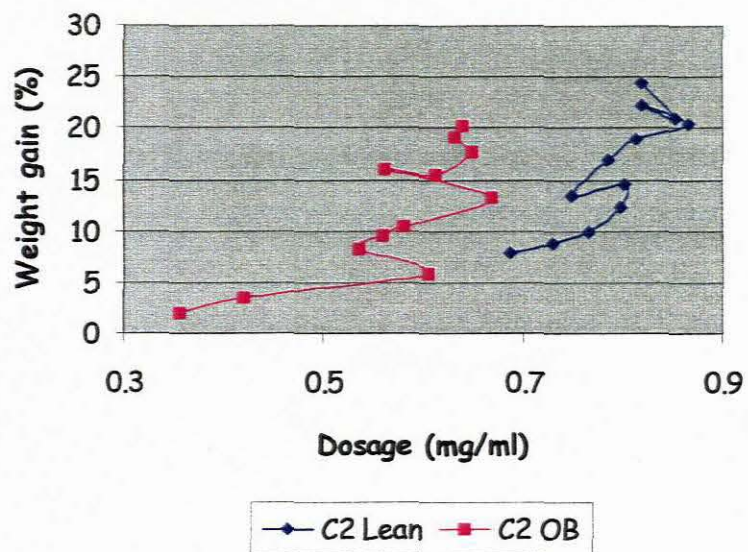


Figure 3.7b: The effect of C2 dosage in weight for animals fed HFD and MD

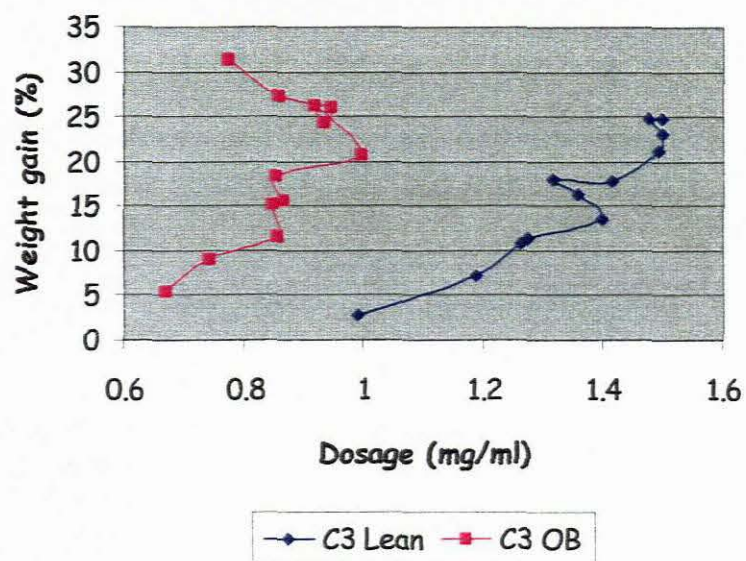


Figure 3.7c: The effect of C3 dosage in weight for animals fed HFD and MD

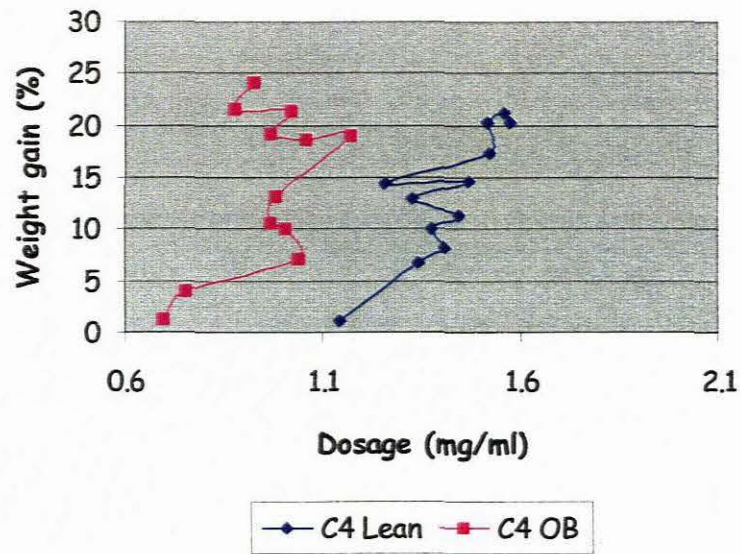


Figure 3.7d: The effect of C4 dosage in weight for animals fed HFD and MD

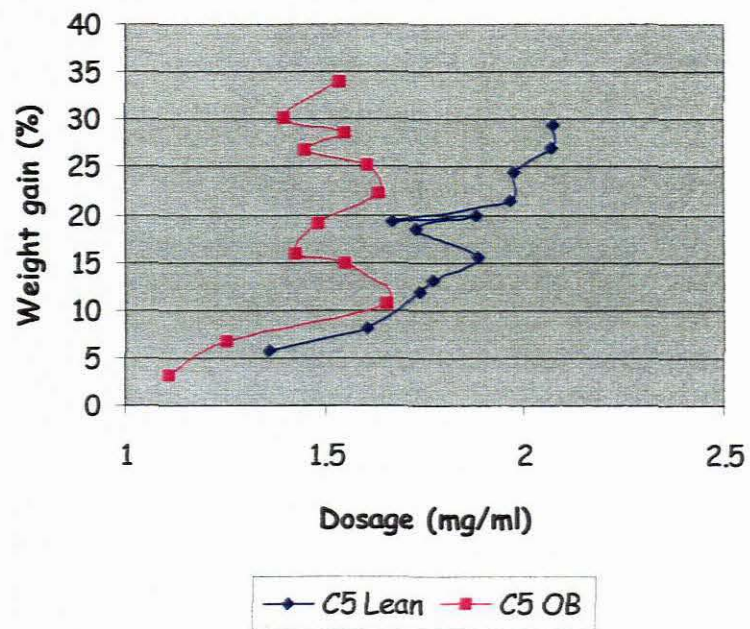


Figure 3.7e: The effect of C5 dosage in weight for animals fed HFD and MD

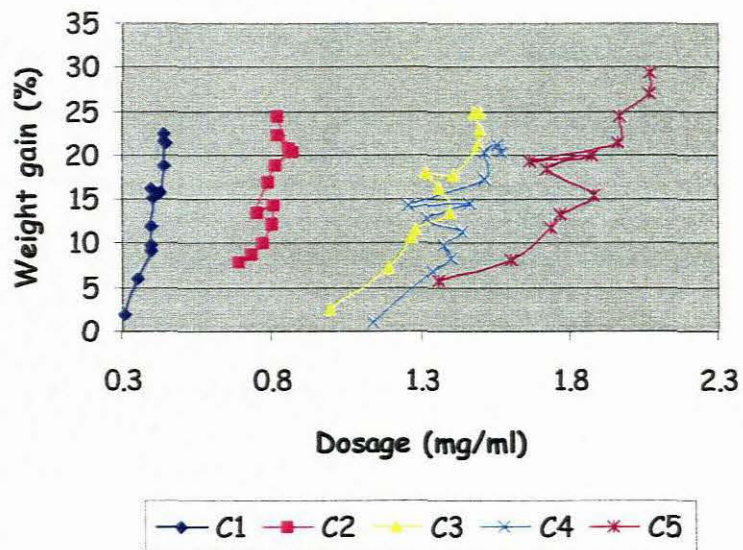


Figure 3.7f: The effect of extract dosage in weight for animals fed MD

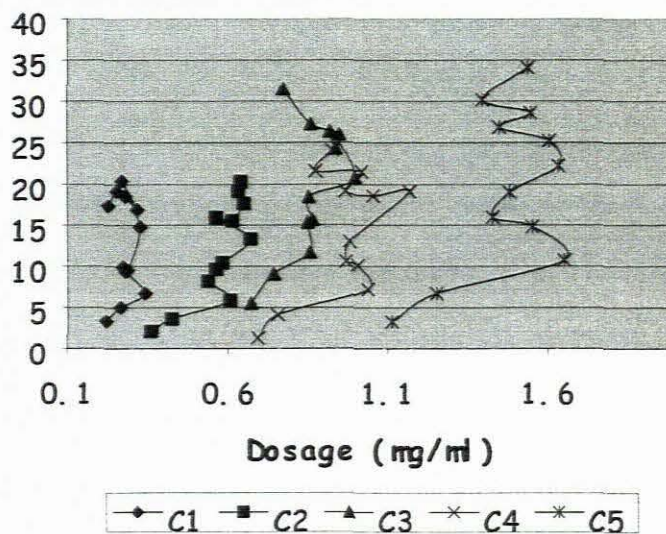


Figure 3.7g: The effect of extract dosage in weight for animals fed HFD

Chapter 4

Discussion

Research on Diabetes treatment and cure is ongoing worldwide. Various metabolic disorders take place when diabetes develops and various drugs are available for treatment of some of these disorders. However these drugs act in ways that cause side effects and other disorders thus perpetuating the condition. Goldstein, (2003) stated two fundamental abnormalities that are involved in the pathogenesis of T2D, that is, resistance to the biologic activities of insulin in glucose and lipid metabolism, and inadequate insulin secretion from the pancreatic β cells. This comes about after many things have happened and the cost of treating these disorders is very high.

In this study an extract from a South African indigenous plant, identified as GUH, was compared with commercially available drugs metformin and rosiglitazone. This was an effort to identify an effective diabetes treatment with reasonable cost for South Africa and the world. It has been known for a number of years that feeding experimental animals diets high in fat leads to insulin resistance (Lavau et al., 1975; Susini et al., 1978). An impaired ability of insulin to stimulate glucose uptake in skeletal muscle with high fat feeding has been demonstrated in both in vivo and in vitro preparations although the mechanism is unknown (Youngren, et al., 2001). Insulin resistance at musculoskeletal level results in hyperglycaemia, increased level of circulating free fatty acids, and compensatory hyperinsulinemia. The development of fasting hyperglycaemia and progression into T2D likely involves impairment in autoregulation of glucose production (Commerford, et al., 2002).

In principle, chronic exposure to HFD could affect variables at any or all of several levels of control and cause obesity. This could include the taste or other sensory qualities of HFD, the processing of fat by the gut, the generation and reception of meal-related signals that control food intake and metabolism. Also the generation and reception by the brain of adiposity indicating signals and brain neurotransmitter systems that regulate food intake and metabolism (Woods et al., 2003). This study showed the groups that were treated with an extract did not differ from the control group, as the levels were different for different weeks. Although there was a slight difference, treatment with an extract showed an increase in food intake. The untreated group, when compared to other groups that were treated and given HFD, showed low food intake. Various reasons can be the cause of this low food intake for the untreated group. Amongst the things that contribute is the administration of sucrose instead of water. This has been reported to lead to high intake of liquid and reduced food intake.

Previous studies showed that plasma glucose plays a prominent role in the short term regulation of food intake, as small spontaneous transient declines in the plasma glucose concentration within the physiological range have been found to induce meal initiation in rats (Louis-Sylvestre and Le Magnen, 1980) and humans (Melanson et al., 1999). It is expected that a sweet solution like that of sucrose will increase the plasma glucose for it is simple sugar and can easily be broken down and accumulate in the blood system. Because this causes plasma glucose to incline the decrease in food consumption takes place in order to overcome the rising plasma glucose levels. The untreated group showed this in the present study. Leptin is believed to also play a role in the food intake but the study of its levels was not included in this investigation.

Different results from the treated groups differed from that of the untreated group. This seemed to be a positive result to that of the study mentioned above about the plasma glucose levels' role in food intake. It is expected that the hypoglycaemic agents will reduce the levels of glucose. The reduction of plasma glucose lead to the increase in the food intake with the aim of bringing plasma glucose levels up. This was seen with the groups that were treated with metformin and rosiglitazone that had higher food intake than the untreated group. The extract treated groups also showed the increased food intake when compared with the untreated group. With the treated groups the intake played a role in determining the weight gain for the groups. As shown in the results the intake of food by the metformin treated group was high also with weight being the highest of all the treated groups. Similarly it was seen with the rosiglitazone treated group consumed a lot more food than the extract treated groups. Campbell and Howlett, 1995 reported findings that metformin reduces or prevents weight gain in obese patients with T2D. Similar findings were reported by Rouru et al., 1992 in animals. The group that was treated with metformin showed similar results by gaining less weight compared to the untreated group, besides the significantly high food intake.

The MD fed groups, compared with the control group, showed to be low in weight for most of the weeks through the treatment period. The difference in weight was not significant for these groups. It was shown earlier that the extract reduced the intake of food for groups that were given MD and treated with extract. This shows a little effect of the extract in normal conditions. It is not clear why the intake for these groups was lower than of the control group. Since many conditions can lead to that at this stage it cannot be said.

The control of rise in glucose is the target for many hypoglycaemic agents. This is delaying the damage that can be due to hyperglycaemia. Hyperglycaemia is recognised as a causal link between diabetes and diabetic complications. The results show the effect of treatment in the rise of blood glucose to be promising in preventing hyperglycaemia. Hyperglycaemia has been reported to have adverse effects on insulin target tissues and on pancreatic β cells, the phenomenon termed glucotoxicity. Chronic hyperglycaemia has been shown to induce multiple defects in β cells, including early decrease in glucose-stimulated insulin secretion and late irreversible changes in insulin-gene transcription and β cell mass (Brownlee, 2003).

Baseline glucose levels showed a difference for the HFD fed groups compared to the control group. These results are likely to be seen in most studies. When the animals are given HFD their glucose levels show to be higher than that of animals fed MD for the same period. In our study the HFD fed groups showed significantly high levels of glucose. This was different after four weeks of treatment, with low levels in the C3 treated group. This group showed a significant drop in blood glucose level that was significantly lower than that of the control, untreated, rosiglitazone and metformin treated groups. The untreated group was also lower than the control group. However the difference was not significant in eight weeks of treatment with the MD fed and HFD fed groups, showing close levels of glucose. This showed to be the effect of treatment since the levels were raised after the twelfth week of treatment. The blood glucose level for the untreated group showed to be the highest. This was expected as the prolonged HFD administration leads to the accumulation of fatty acids in the blood stream. The prolonged intake of HFD also leads to insulin resistance and increased glucose and insulin levels show insulin resistance.

Insulin levels were significantly different for the HFD compared to MD fed groups after twelve weeks of feeding before treatment (baseline). This indicates similar results shown by the studies that used HFD to feed animals with MD as a control. Rats diets enriched in fat or sucrose reduce the ability of insulin to suppress glucose production in vivo and reduced the ability of insulin to suppress gluconeogenesis in perfused livers and isolated hepatocytes (Commerford, et al., 2002). This causes more insulin to be produced to overcome the resistance leading to high insulin and high glucose levels. The insulin levels for the MD fed groups showed no significant difference after eight weeks of treatment and a sharp rise in the last week. This rise was less for the treated groups than that of the control. This can be due to the sensitivity that the treated animals have for insulin due to the fact that the glucose levels did the same during the last week.

The HFD fed groups showed a different insulin level to that of the MD fed groups. The group that was treated with C4 showed increasing insulin levels more than all the other groups. This was even more in the eighth week of treatment where the entire treated groups and the untreated group had dropped insulin levels lower than in the baseline. This can be due to the poor effect that the extract has at this concentration. But with groups on HFD treated with extract the effect of extract in the last week of treatment showed the increase of insulin production to be dependent of the concentration. The C4 and C5 treated groups showed higher insulin levels than the other extract treated groups in lower concentrations. It is not clear why the levels of insulin increase for the extract treated group during the last week. But the results as shown earlier, with a decrease in insulin for these groups show that the sensitivity can

be of importance as an action for the extract. The possible mechanism of the extract can be increasing insulin sensitivity in target sites and increasing the B-cell volume with time.

Metformin and rosiglitazone acted in a similar manner to the extract. The insulin levels for the groups that were treated with metformin and rosiglitazone dropped from baseline levels to low insulin levels on the eighth week of treatment. Although the rosiglitazone treated group's insulin levels rose in the last week was low compared with that of the extract treated groups on C3 and higher concentrations. The metformin treated group had the lowest insulin levels during the last week of treatment. The studies that have been done on metformin show that it acts as an insulin sensitising agent, lowering fasting plasma insulin concentration by inducing greater peripheral uptake of glucose, as well as decreasing hepatic output (Nisbet, et al., 2004). This caused metformin in the present study to have less insulin and glucose levels when compared to other groups. When looking at the levels of glucose for the extract treated animals it shows that although metformin brings down the insulin level the extract is good in controlling the rise in glucose. The extract mode of action can be insulin targeted because the insulin levels for groups treated with it becomes high, with low glucose levels which show no resistance but increased output. The rosiglitazone group showed almost the same level of insulin but very high glucose levels. This may be due the resistance of the animals treated with rosiglitazone to insulin with time. In the last week of treatment it showed that the HFD fed groups treated can control their glucose levels and the insulin levels for these animals were lower than that of the control group. The rosiglitazone treated group showed high

glucose but low insulin, unlike the untreated and C4 group that had insulin and glucose elevated.

Treatment of MD fed groups did not make a significant difference in serum cholesterol of the treated groups compared to the control group. This was shown in the total cholesterol. This may be the reason that the extract does not have a noticeable effect in lipid levels of normal rats. The MD fed groups when compared to the control had a little lower total cholesterol in the groups that were treated with C1 and C5. The difference is not huge but there is an indication that the extract may have an effect on the fat metabolism. The lipid clearance plays a role in plasma lipid levels more than the produced lipids. Triglyceride levels were not significantly different for the groups that were treated, compared to the control, for the groups that were fed MD. But highly concentrated C5 showed slight lower levels than the control group. This may be as a result of enhanced lipid clearance. Except for the group that was treated with C1 the levels of HDL showed no difference. LDL levels of treated groups showed to be lower than that of the control group. Results like this show this to be a positive attempt of reducing the risk factors of developing chronic lifestyle diseases like coronary heart diseases (CHD) and diabetes.

Total cholesterol for the control group compared to that of some groups that were fed HFD differed. Feeding animal HFD has been reported to increase the cholesterol levels. The untreated group showed no significant difference to the control group in total cholesterol. The metformin, C1 and C2 groups also had no significantly different cholesterol levels. Treating animals with rosiglitazone, C3, C4 and C5 showed these treatments to reduce the cholesterol levels, as was seen when compared to the control

group. The action by the extract and rosiglitazone can be similar. Metformin was reported to increase the flowing cholesterol because it also enhances the glucose uptake by speeding up beta-oxidation. In our study this was shown by high total cholesterol in the metformin treated group.

Triglycerides are a risk factor in the development of coronary heart disease through atherosclerosis. The untreated group showed higher TAG levels than any other group in our study. For an unknown reason the group that was treated with C4 also showed high TAG levels. Groups that were treated with extract C1-C4 showed to have significantly high TAG levels. This can be so because of poor clearance of fatty acids in the blood stream. The other reason that can contribute partly could be the breakdown of fat deposits and that causes the accumulation of blood TAG. Metformin, rosiglitazone and C5 showed no significant difference to the control group in TAG levels. The mechanism of action for these three treatments in reducing the levels of TAG is unknown besides the studies that have already taken place for metformin and rosiglitazone.

High levels of HDL have been recommended for good health and reduced risk of developing disease, including diabetes. Treatment of animals with an extract resulted in low HDL levels. The reason for this is not clear but the untreated group and the groups treated with metformin and rosiglitazone showed no significant difference compared to the control group. Significantly low HDL levels for extract treated groups can have less effect to the groups treated with it. As was seen earlier with total cholesterol, which has been low compared to that of control, low HDL levels are still corresponding with total cholesterol. These results show that the extract has an ability

to reduce cholesterol levels. This can be caused by blockage of fat absorption through enzyme deactivation.

The rosiglitazone treated group showed significantly low LDL levels compared to the control group. This is proving to be a strong mechanism of action since the HDL was not significantly different and the LDL differed significantly. The ratio of HDL: LDL will somehow be high which is good. The rest of the groups showed no significant difference to the control group. Metformin showed high LDL also which is already said in previous studies to increase LDL levels through the speeding up of beta-oxidation.

But the HFD fed groups when compared to each other showed a difference. The group that was treated with metformin showed high levels of total cholesterol compared to the untreated, rosiglitazone treated group and all extract treated groups. But this was not linked with the circulating plasma TAG. The metformin treated group showed significantly low levels of TAG compared with the untreated group. The group that was treated with C4 also showed significantly higher TAG levels than the metformin treated group. The reason for high levels of TAG in this group is not clear. Rosiglitazone and C5 treated groups also had low TAG levels compared with the untreated group. Although other extract treated groups had no significantly different TAG levels their levels were low compared to the untreated group.

HDL and LDL levels are also used as markers for the development of diabetes complications. The ratio of the two is an indicator of whether an individual is at risk or not. The total cholesterol was low in extract treated groups, as shown earlier. This led to low HDL levels that are significantly low for the extract treated group when

compared to the metformin treated group. Rosiglitazone also showed significantly high levels of HDL compared to C1, C2 and C3. The groups that were treated with high concentration of an extract, that is C4 and C5, although they showed low HDL, the difference was not significant compared to the untreated, rosiglitazone and metformin treated groups.

LDL levels for the rats that were treated with metformin were significantly higher than those that were treated with highly concentrated extract (C4 and C5). Although the metformin treated group's LDL levels were not significantly higher than that of other animals treated with lower extract concentrations it was the highest of all treated groups and the untreated one. The reason for this can be metformin's ability to form the LDL because the TAG were less, suggesting that the fatty acids bond with lipoproteins rather than to form triglycerides. The rosiglitazone treated group showed very low LDL levels compared to the metformin treated group. This suggests the action of rosiglitazone to be in enhancing the storage of fats instead of breaking them down into the blood stream. The rosiglitazone group also had significantly low levels of LDL than the C1 and C2 treated groups and the untreated group.

Glucose clearance differed amongst the groups. The MD fed groups showed a different glucose clearance percentage. Treated groups were clearing glucose levels faster, especially the groups that were treated with C2 and C4. But the difference in glucose clearance was not significant for MD fed groups compared to the control. The clearance of glucose was low for the groups that were treated with C2 and C4 for HFD and not for MD fed groups. When the control group was compared to HFD fed groups the clearance of glucose was faster for the group treated with C5. The

metformin treated group also showed higher clearance of glucose than the control group. Glucose clearance by the C1 and C3 groups was also high for the animals that were treated with the extract and fed on HFD.

The effect of dosage to weight gain by the animals that were treated with an extract showed to be increasing with increase in concentration. The increase of concentration led to the increase in dosage and hence the percentage weights gain. This was caused for the extract because the animals treated with an extract got different concentrations. The dosage for the animals treated with a low concentration of an extract was low and hence the weight gain. This may be related to the intake of food that was low in these groups. Increasing the dosage has been recommended even in commercially selling drugs like metformin. This may be because the increase in dosage means a stronger drug.

Many people have used metformin and rosiglitazone for the treatment of diabetes. The extract that was used for this study showed almost similar functions as the above mentioned drugs. Amongst the risk factors in developing diabetes is obesity. The extract showed increased food intake similar to that shown by the drugs. Kang et al., (2004) reported that when greater quantities of energy (in the form of food) than can be expended enter the body, body weight increases. The results of this study for treated groups showed higher intake with increase in body weight but the increase in body weight was not as high as that of the untreated group with low intake. The treated groups had high body weight according to the way they were taking in food, leading to the extract treated group weighing the least of treated groups and the heaviest being the metformin treated group that had the highest intake of food. This shows some advantage for the extract as a treatment for obesity a risk factor for

diabetes. For the groups that were treated with extract the concentration played a role in weight gain with the groups given the lowest concentration weighing the least. The molecular mechanism of the extract increasing the food intake remains unknown and creates a gap in the present study. Although the extract was used in this study to treat MD fed groups metformin and rosiglitazone were not used to treat these animals. The extract showed different results for HFD compared to MD. There was a decrease in food intake for the groups treated with the extract and fed MD that led to less body weight than the control. Paolisso, et al., (1998) reported the inhibition of food intake and lowering of body weight and body fat by metformin in obese non-diabetic patients and that can be the case with the extract in the present study.

All treatments brought the high glucose levels down after the significant difference that was shown by the groups before treatment. The groups that were treated showed no significant difference to the MD fed group after four weeks of treatment. The group that was treated with C3 showed an increase in insulin and a significant decrease in glucose levels. This different action by C3 in other concentrations is not clearly known. The use of teas for the same purpose as extract in other experiments showed that the increase of tea catechins increases the effect that the tea has. The results that the plant extract showed in controlling the glucose and insulin levels showed positive results for the extract as a hypoglycaemic agent. The levels of insulin and glucose rose for all the groups in the last week of treatment. But this rise was better for the treated groups, except for the rosiglitazone and C4 treated groups. The rise in glucose levels is not clear but because all the groups that were used in this experiment showed this increase it could be due to the prolonged time of experiment. The control and the untreated groups showed the increase in both insulin and glucose

levels. These changes led to the treated groups having high but better levels compared to untreated groups. The control of rising glucose and insulin levels shown by extract treated groups was even better than that of the rosiglitazone group during the last week of treatment.

Glucose clearance is playing a role in the control of glucose accumulation in the blood. This ability varied between groups in the present study. For groups that were treated with an extract and fed MD only the group treated with C1 showed lower clearance than the control group. Except for this group all the groups had high glucose clearance. This ability of increasing glucose clearance in the blood by the extract needs to be studied in detail. The drugs that have been used so far have been found to increase the tolerance of glucose through a number of mechanisms. The known drug metformin increases sensitivity of receptors to insulin amongst its actions in treating diabetes. Other drugs block the enzyme activities leading to inhibition of absorption in the intestines like alpha glucosidase inhibitors, with some drugs stimulating the pathways to take place, like rosiglitazone. This increased glucose clearance is also showing the positive results for the extract. But the concentrations of the extract somehow show different abilities and this is not explained since the contents of the plant extract were not studied as part of the project. The animals treated with C2 and C4 showed high clearance of glucose in the MD fed group but their insulin levels were also higher than that of other groups which may be the cause of the fast glucose clearance rate.

The HFD fed groups also showed increased clearance of glucose by the extract treated group that was fed C5. This showed to be the highest clearance of all the groups that were used for the study. But the insulin levels for the groups that were treated with

extract rose with that of other groups in the last week. The glucose levels of these groups, although they rose also, their levels seemed to show sensitivity since the glucose levels were lower than that of other treated groups. This may be the action of an extract to increase insulin levels while increasing the sensitivity of the receptors to insulin. Metformin but not rosiglitazone showed this also but the glucose level for metformin was high compared to that of the extract.

The extract showed a bigger decrease in the levels of cholesterol compared to other treatments. The total cholesterol of the control group was high compared to that of the treated MD fed groups. This was not a significant difference but showed to be one of the abilities that the extract has. The extract concentration C3 and C5 showed a better clearance of triglycerides. These are the two concentrations that showed a similar level with the control group, with other groups showing slightly higher triglycerides. Since it has been shown earlier that even the body weight decreases in these animals this shows that the lipids are not cleared from the blood to be stored. The control of fat levels by the extract is not known but it can be due to the inhibited absorption of lipids in the intestines or reduction in the activities of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase that is a rate-limiting enzyme in cholesterol biosynthesis.

The extract also showed the difference when compared to other treatments for HFD fed groups. The total cholesterol for the groups treated with extract was significantly low compared with metformin. When compared to rosiglitazone and the untreated groups the cholesterol for these groups was also higher than that of extract treated groups but the difference was not significant. Because of low total cholesterol this led

to low levels of HDL for the extract treated groups, with low LDL levels. But the highest concentration of an extract showed good cholesterol control with high HDL, low LDL and triglycerides. The group that was treated with C5 showed positive results and a good control of a condition that may lead to diabetes development. Although other concentrations of extract were showing good results in control of some conditions they were lacking the ability of bringing other conditions down. The action of the extract at concentration C4 showed poor control of glucose levels and showing insulin resistance, especially in the last week of treatment.

Extensive work has been done with the drugs namely metformin and rosiglitazone. The use of the extract in this study was without a change in diet and the use of exercise. The extract showed the difference in rats treated to be even better than that of the drugs in some cases. The highlighted advantage of the extract use is the ability that the extract has in reducing the blood cholesterol and the reduction of blood glucose levels. What was shown by all the treatments is the reduction of their effects with time. This was shown by all the treatments but with less difference in the ability of the extract, especially when used to treat HFD fed groups. Although the extract showed even better results, compared to the commercially available drugs, in some cases a lot more studies need to be done.

Food and exercise have been used with drugs in most of the studies. Maybe better results can be found even with the extract used for this study if the diet can change. The results were better for some HFD fed animals treated with extract even when compared to the animals that were treated with extract and fed MD. This alone showed that it does not depend on the diet used and it can result in better results even

when the food used is high in fat content. The number of animals that were used for this study was high, maybe in future the animals can be split and exercise used to work with extract to see the contribution of exercise with treatment. The molecular content of the extract needs to be studied in detail to find what contributes the effect that the extract has in the control of increasing levels of insulin, glucose and lipids. The results that were found from study in animals showed to be positive for the extract, even more than drugs in some parameters.

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