INVESTIGATION OF THE POSSIBLE ANTI-DIABETIC ACTIVITY OF *ICACINA TRICHANTHA, ANANAS COSMOS AND URARIA PICTA IN A RAT MODEL*.

A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand in fulfilment of the requirements for the degree master of Science in Medicine

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Declaration

I, Femi Kayode Fatokun declare that this research report is my work. It is being submitted for the degree of Master of Science in Medicine in the department of Pharmacy and Pharmacology in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Signature of candidate.

22 Day of October, 2010

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Abstract

Natural remedies from medicinal plants are considered to be effective and safe alternative treatment for diabetes mellitus. The aim of this study was to demonstrate the hypoglycaemic and antidiabetic activity of the aqueous extract of *Icacina tracantha (tuber) (fam Icacinaceae)Ananas cosmos* (*fam. Bromeliaceae)*and *Uraria picta (*leaves*) (fam leguminosae)* on an animal model of insulin resistance, a condition which predisposes to type 2 diabetes. The plants have a long history of use as anti-diabetic agents in western Nigeria.

Method: 120 male Sprague-Dawley rats were assigned into two major groups. One group was fed on normal rat chow with the other group fed on a high calorie diet for four months a period sufficient for the animals to be fed to attain insulin resistance. The animals were then randomly assigned into different groups (each containing 6 male rats). The plant crude extracts were made by weighing specific dried quantities of each plant, boiling in distilled water for about 2 hours, cooling overnight and separating solid from liquid by filtration. The solution was then poured into pre-weighed 250 ml beakers and allowed to dry in an oven at a temperature of 60^{o}C. The dried, crude extracts were then weighed out and required doses prepared from the extracts. A non-treated group of animals was used as the control. The mixed dose of extract was administered at 300 mg/kg. Over a 3 week period, all the animals were orally dosed with the different doses of plant extracts daily while metformin was administered through the animals' drinking water, blood was collected from the tail vein of each rat prior to dosing and thereafter weekly, plasma was preserved and

analysed for glucose, insulin, free fatty acid concentrations and calculation of HOMA values to determine insulin sensitivity. During this period, the animals were weighed weekly and food intake was measured every three days. An oral glucose tolerance test (OGTT) was performed after the dosing period and fasting, 0, 30, 60 and 120 minute blood samples were taken and assayed for glucose concentration. Animals were terminated and blood analysed.

Statistical analysis: The results were tabulated as mean ± standard deviation and percentage median ± quartile range. The statistical analysis for other parameters was carried out via ANOVA (between groups) and Student's paired T test (within groups). Only data from percentage median and quartile range was used because of the observed variation in glucose concentration between groups even at baseline values. Statistica software (StatSoft, Tulsa, OK, USA) was used for the analysis.

Results: All plant extracts in the study showed differing concentration of significant difference in their effect on the plasma glucose, insulin and free fatty acid concentrations in the rat. The most significant effect was observed on the insulin concentration in the normal rat chow and high calorie diet fed animals. The plant extracts were observed to improve insulin sensitivity in most of the groups. This effect was more significant in the normal rat chow fed rats. The effect of the plant extracts on the weight, food consumed glucose and free fatty acid was minimal and in most of the groups was not significant.

Conclusion: In conclusion, the results obtained suggest that the plant extracts may be used to improve insulin resistance in the management of diabetes mellitus.

Abbreviations

W.H.O	World Health Organization
TM	Traditional medicine
I T	*ICACINA TRICHANTHA*
U P	*URARIA PICTA*
A C	*ANANAS COSMOS*
O. G. T. T	Oral glucose tolerance test.
SD	Sprague Dawley
OD	Optical density
µL	Micro liters
ml	Milliliters
nm	nanometers
^{0} C	degrees centigrade.
rpm	revolutions per minute
HOMA	Homeostatic model assessment
ADA	American Diabetes Association
IDF	International Diabetes Federation.
ATM	Alternative and Traditional Medicine
DM	Diabetes Mellitus.
HCD	High calorie diet

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CHAPTER 1. PREFACE.

Evidence from fossil records date human use of plants as medicines at least to the Middle Paleolithic age some 60,000 years ago (Solecki and Shanidar, 1975). The earliest known medical document is a 4000-year-old Sumerian clay tablet that recorded plant remedies for various illnesses. The ancient Egyptian Ebers papyrus from 3500 year ago lists hundreds of remedies.

Since ancient times, plant remedies have been used to help relieve diabetes. In the 6th century B.C., Sushruta, an Indian physician classifying diabetes as a urinary disorder recommended plant remedies for its treatment and *Gymnema sylvestre* was advised for its treatment. (Shanmugasundaram et al, 2005).

Medicinal plants are an important element of the indigenous medical systems in different parts of the world and plants have long provided mankind with a source of medicinal agents, with natural products once serving as the source of all drugs and in recent times as a major source of novel medicines in the pharmaceutical industry. (Ephraim et al, 2008).

Different cultures have developed peculiar forms of traditional medical systems with medicinal plants for treating a number of diseases, including diabetes. In Ayurveda, Siddha and Unani systems of medicine, several herbs or plant products have been used for the treatment and management of diabetes mellitus (DM) (Raju et al, 2006). Though conventional synthetic drugs have made considerable progress in the

management of DM, traditional plant treatments for DM are also being used throughout the world and the search for natural anti-diabetic plant products for controlling DM is on-going. (Raju et al, 2006).

Diabetes is a serious condition for the individual and society. Its rapidly increasing global prevalence is a significant cause for concern. The prevalence of diabetes throughout the world has increased dramatically over the recent past, and the trend will continue for the foreseeable future. One of the major concerns associated with diabetes relates to the development of micro- and macro-vascular complications, which contribute greatly to the morbidity and mortality associated with the disease. Progression of the disease from the pre-diabetic state to overt diabetes and the development of complications occur over many years (Cefalu, 2006). The prevalence of diabetes is higher in developed countries than in developing countries, but the latter will be hit the hardest by the escalating diabetes epidemic. Increased urbanization, westernization and economic development in developing countries have already contributed to a substantial rise in diabetes. The prevalence of diabetes in adults is projected to rise in both developed and developing countries. While diabetes is most common among the elderly in many populations, prevalence rates are significantly rising among comparatively young and productive populations in the developing world (Website reference 1).

The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030. These findings indicate that the “diabetes epidemic” will continue even if the prevalence of obesity remains constant. Given the increasing prevalence of obesity, it is likely that these figures provide an underestimate of future diabetes prevalence (Sarah et al, 2004). The epidemic nature of diabetes continues to affect ever-increasing numbers of people around the world while public awareness remains low. (Website reference 2).

Various ethno-pharmacological surveys have shown that a number of medicinal plants have been used for the treatment of diabetes with various authors attesting to the efficacy of the plants in the control of both type 1 and II diabetes (Villasenor and Lamdrid, 2006; Maryna et al 2008).

Although medicinal plants have been historically used for diabetes treatment throughout the world, few of them have been validated by scientific criteria (Medeiros et al, 2008). Numerous medicinal plants and herbal preparations have been shown to reduce blood glucose concentration through various mechanisms, although they are usually limited by toxicity or relative lack of efficacy compared with standard medications (Seham et al, 2006).

The medicinal plants investigated in this study, *icacina trichantha, ananas cosmos and uraria picta* have been used in Western Nigeria for managing diabetes(personal

observation at Complete Cure HerbalClinic, 19 Igbobi College Road, Jibowu, Lagos) but without any scientific validation to confirm their anti-diabetic effects.

The aim of this study wasto investigate the use of *Icacina tricantha* , *Uraria picta* and *Ananas cosmos* individually and in combination to lower blood glucose, insulin andfree fatty acid concentrations and improve insulin sensitivity in a rat model of insulin resistance.

The study objectives included the following:

- To determine the ability of the individual plant extracts to improve insulin sensitivity in Sprague Dawley (SD) male rats fed a high calorie or a normal rat chow diet. Insulin sensitivity will be determined by using the HOMA method: level of insulin resistance = [fasting insulin (μU/mL) × fasting glucose (mmol/L)]/22.5.
- To determine the efficacy of the combination of the plant high calorie diet AC and *Uraria picta* extracts to improve insulin sensitivity in the animals.
- To confirm the effect of the plant extracts on the biochemical parameters of insulin production, glucose lowering, and lowering of serum free fatty acid concentration.
- To compare the anti-diabetic effect of the various plant extracts against a commonly used commercial therapy (metformin).

A number of animal models have been employed to test and confirm the effect of medicinal plants on induced diabetes. In this research work, a rat animal model was

used. The model was characterised by first feeding the animals on a high calorie diet for a period of four months to induce insulin resistance.

The animals were then given the plant extracts orally for three weeks, weighed weekly, while blood samples were also collected weekly through the tail vein. An OGTT was performed after the period of three weeks following which the animals were terminated and blood samples collected.

The OGTT is a diagnostic test that measures the body's ability to metabolise glucose. It was performed to observe the effect of the plant extracts on glucose metabolism in the rats. Where the plants extracts had a positive effect on glucose metabolism the clearance rate was greater, and this is shown by measuring the area under the curve obtained from glucose readings taken over a 2 hour period. The plant extracts that have a better glucose clearance rate had a lower area under the glucose curve when compared to the control, untreated animals.

Further analysis to ascertain the effect of the individual plant extracts, the combination of the extracts, metformin and non-treatment in all the groups of animals for the three week dosing period was carried out using weekly blood sample collected. The samples were analysed for glucose, insulin and free fatty acids. These are biochemical parameters that determine the effect of the plant extracts on diabetes.

The first chapter contains the introduction, background to study, prevalence of diabetes, aims and objectives of the study, methodology and rational of studies. This chapter describes the age long use of medicinal plant to treat different kinds of diseases, including diabetes. The chapter documents information showing the increasingly growing threat of diabetes, the possibility of finding alternative methods of treatment using medicinal plants, and the method to be employed in this study to achieve the aims listed.

Chapter 2 defines the types and classification of diabetes mellitus and looks into the history of the disease from the time of its documented discovery, the economic burden associated with the disease, financial strains on nations and the association between diabetes and insulin resistance. The chapter further sheds more light on the growing influence of traditional medicine as a credible means of treating and managing diseases including diabetes. Furthermore, the chapter sheds more light on the rational for using the plants used in the present study, their history of use and literature review on these plants.

The third chapter describes the materials, methods equipment and reagents used in the present study to investigate the possible anti diabetic effect of the medicinal plants using a rat model, based on feeding the animals on a HCD for four months.

Chapter 4 is a composition of the results and data presented in tables and graphical figures. The chapter also describes the results obtained in the studies.

Chapter 5 contains the discussion on the outcome of the results obtained, the effects of the different medicinal plants on the biochemical parameters that affect diabetes including, plasma glucose concentration, weight of animals, food consumed, plasma insulin concentration, HOMA values, oral glucose test and FFA.

Lastly, the 6th chapter documents the conclusion from the results obtained in the studies and inference drawn from the studies.

CHAPTER 2. DEFINITION, HISTORY, TYPES, CONSEQUENCES AND ECONOMIC BURDEN OF DIABETES

2.1 Diabetes mellitus

Diabetes mellitus (DM) is a condition primarily defined by the level of hyperglycemia giving rise to the risk of both micro- and macro-vascular damage. It is associated with reduced life expectancy, significant morbidity due to specific diabetes related microvascular complications (retinopathy, nephropathy and neuropathy), increased risk of macrovascular complications (ischaemic heart disease, stroke and peripheral vascular disease), and diminished quality of life. It is a metabolic disorder characterized by resistance to the action of insulin, insufficient insulin secretion, or both **(**Moore et al., 2004, WHO/IDF consultation, 2006).

2.1.2 History of diabetes.

The first observations of high glucose content in urine was made by early healers around 1500 BC while the first known recorded documentation of the disease was mentioned on a 3rd Dynasty Egyptian papyrus by the physician Hesy-Ra who mentions polyuria (frequent urination) as a symptom.

Over the centuries, a number of early physicians have described the disease by its several symptoms. Diabetes had no known cure or means of effectively treating diabetic patients until 1922 when experimental treatment with insulin was considered a success following the treatment of a 14 year old diabetic patient, by Dr. Banting,

Prof. Macleod and Dr .Collip, who were awarded a Noble prize in 1940. Further research led to the discovery of oral anti-diabetic agents in 1955. Over the years, the number of diabetic patients have been on a steady increase with the World Health Organization (WHO) estimating that about 300 million people will have the disease by the year 2030. (Website reference 3).

2.1.3 Types of diabetes.

The vast majority of diabetic patients are classified into one of two broad categories:

Type 1 diabetes, also known as insulin-dependent diabetes which is caused by an absolute deficiency of insulin, Type 1 diabetes is characterized by a lack of insulin production. Without daily administration of insulin, Type 1 diabetes is fatal. Symptoms include excessive excretion of urine (polyuria), thirst (polydipsia), constant hunger, weight loss, vision changes and fatigue. These symptoms may occur suddenly. Type 1 diabetes is an autoimmune disease in which the insulin secreting - cells of the pancreas are destroyed by the immune system.

Type 2 diabetes, also known as non-insulin-dependent diabetes is characterized by the presence of insulin resistance with an inadequate compensatory increase in insulin secretion. Type 2 diabetes results from the body's ineffective use of insulin. Type 2 diabetes comprises 90% of people with diabetes around the world, and is largely the result of excess body weight and physical inactivity. Symptoms may be similar to those of Type 1 diabetes, but are often less marked. As a result, the

disease may be diagnosed several years after onset, once complications have already arisen. Until recently, this type of diabetes was seen only in adults but it is now also occurring in obese children (Weiss et al, 2004Pinkney, 2002).

In addition, women who develop diabetes during their pregnancy are classified as having gestational diabetes. Symptoms of gestational diabetes are similar to Type 2 diabetes. Gestational diabetes is most often diagnosed through prenatal screening, rather than reported symptoms and is associated with an increased risk of Type 2 diabetes (Kim et al 2002).

There are also a variety of uncommon and diverse types of diabetes which are caused by infections, drugs, endocrinopathies, pancreatic destruction, and genetic defects. These unrelated forms of diabetes are classified separately (WHO Fact sheet N°312, 2005, James et al, 2002, Website reference 4).

2.1.4 Consequences of diabetes

In 2005, an estimated 1.1 million people died from diabetes. Almost 80% of diabetes deaths occur in low and middle-income countries. Almost half of diabetes deaths occur in people under the age of 70 years; 55% of diabetes deaths are in women. The WHO projects that diabetes deaths will increase by more than 50% in the next 10 years without urgent action. Most notably, diabetes deaths are projected to increase by over 80% in upper-middle income countries between 2006 and 2015. Over time, the hyperglycaemia associated with diabetes can lead to damage to the heart, blood vessels, eyes, kidneys, and nerves (Ahhassh et al, 1995).

- Diabetic retinopathy is an important cause of blindness, and occurs as a result of long-term accumulated damage to the small blood vessels in the retina. After 15 years of diabetes, approximately 2% of people become blind, and about 10% develop severe visual impairment (WHO Fact sheet N°312, 2005).
- Diabetic neuropathy is damage to the nerves as a result of diabetes, and affects up to 50% of people with diabetes. Although many different problems can occur as a result of diabetic neuropathy, common symptoms are tingling, pain, numbness, or weakness in the feet and hands.Neuropathy in the feet increases the chance of *foot ulcers* and eventual *limb amputation*. (WHO Fact sheet N°312, 2005).
- Diabetes is among the leading causes of kidney failure: 10-20% of people with diabetes die of *kidney failure*(WHO Fact sheet N°312, 2005).

- Diabetes increases the risk of *heart disease and stroke*: 50% of people with diabetes die of cardiovascular disease (primarily heart disease and stroke) (WHO Fact sheet N°312, 2005).

2.1.5 The economic burden of diabetes

Diabetes and its complications impose significant economic consequences on individuals, families, health systems and countries. WHO estimates that over the next 10 years (2006-2015), China will lose $558 billion in national income due to heart disease, stroke and diabetes alone (WHO Fact sheet N°312, 2005).

The American Diabetes Association (ADA) estimated that the national costs of diabetes in the USA for 2002 was $US 132 billion, increasing to $US 192 billion in 2020 **(**WHO/IDF consultation, 2006).

2.1.6 Insulin resistance, hyperinsulinaemia and Type 2 diabetes

Insulin resistance is the condition in which there is reduced sensitivity to normal concentrations of insulin, thus resulting in a situation where normal amounts of insulin are inadequate to produce a normal insulin response from fat, muscle and liver cells (Akbarzadeh et al, 2007). Insulin resistance in fat cells results in hydrolysis of stored triglycerides due to the reduced ability of insulin to inhibit lipolysis, which elevates free fatty acids in the blood plasma. Insulin resistance in muscle reduces glucose uptake, whereas insulin resistance in liver reduces glucose conversion to glycogen and blunts the ability of insulin to inhibit gluconeogenesis, with these effects serving to elevate blood glucose.

Although the primary factors causing Type 2 diabetes are unknown, it is clear that insulin resistance plays a major role in its development. (Shulman, 2000; Felber and Golay, 1995).

Evidence for this comes from the presence of insulin resistance 10 – 20 years before the onset of the disease, from studies demonstrating that insulin resistance is a consistent finding in patients with Type 2 diabetes and, studies demonstrating that insulin resistance is the best predictor of whether or not an individual will become diabetic or not (Warram et al, 1990).

This state described as “insulin resistant syndrome, metabolic syndrome or syndrome X” is characterized by abnormalities in glucose and lipid metabolism, obesity, high blood pressure and Type 2 diabetes. The causes of metabolic syndrome are extremely complex and have only been partially elucidated. However, metabolic syndrome is a progressive disorder, with a major outcome being Type 2 diabetes. Interventions that slow the rate of progression commonly reduce insulin resistance, supporting the importance of this parameter in disease progression. (Shulman, 2000).

2.1.7. The aetiology of obesity-induced insulin resistance.

Obesity is a major cause of type 2 diabetes (T2DM) in all affected populations, however, obesity is neither sufficient nor necessary to develop T2DM or cause insulin resistance (Tataranni, 2002). Even though the exact aetiology of obesity is not known, it is widely accepted that the disease is caused by an imbalance of energy

intake and expenditure in the body, where more energy is taken in and stored than is expended.(Roberts et al, 2004). Adipose tissue or body fat or just fat is loose connective tissue composed of adipocytes. It is technically composed of roughly only 80% fat. Obesity dose not just set in when there is excess weight but it is best considered as being an increase in adiposity involving an increase in adipose tissue mass. (Coppack, 2005)

Adipose tissue is not just a collection of fat with the role of insulating and protecting the body. It has been established that adipose tissue is an endocrine organ involved in secreting a number of bioactive peptides and factors. These factors and peptides include leptin, angiotensinogen, cytokines, adiponectin,complement components, plasminogen activator inhibitor-1, proteins of the renin-angiotensin system, and resistin.(Kershaw et al, 2004, Ahima et al, 2006).

The important endocrine function of adipose tissue is emphasized by the adverse metabolic consequences of both adipose tissue excess and deficiency. Cytokines like TNF alpha and interleukin 6 (IL-6) have been implicated in the pathogenesis of obesity and insulin resistance. Adipose tissue expression of TNF is elevated in obese rodents and humans and is positively correlated with obesity and insulin resistance (Lorenzo et al, 2007, Ruan et al, 2003)

In rodents, resistin can induce insulin resistance. Adipose tissue IL-6 expression and circulating IL-6 concentrations are positively correlated with obesity, impaired glucose

tolerance, and insulin resistance. Furthermore, plasma IL-6 concentrations predict the development of type 2 diabetes and cardiovascular disease. Peripheral administration of IL-6i nduces hyperlipidemia, hyperglycemia, and insulin resistance in rodents and humans. IL-6 also decreases insulin signalling in peripheral tissues by reducing expression of insulin receptor signalling components. IL-6 also inhibits adipogenesis and decreases adiponectin secretion. (Tiziana et al, 2006)

Adiponectin is highly expressed in adipose tissue, and circulating adiponectin concentration is decreased in subjects with obesity-related insulin resistance and type 2 diabetes. Adiponectin inhibits liver gluconeogenesis and promotes fatty acid oxidation in skeletal muscle. In addition, adiponectin counteracts the pro-inflammatory effects of TNF-alpha on the arterial wall and probably protects against the development of arteriosclerosis (Bastard et al, 2006, Wassink et al, 2007).

2.1.8. Visceral obesity intra-muscular fat deposition and insulin resistance.

Regional fat distribution is a risk factor for the development of type II diabetes and insulin resistance. Obese individuals with high levels of visceral fat are at a higher risk of developing both type 2 diabetes and insulin resistance. (Després, 1993)

Visceral obesity is a form of obesity due to excessive deposition of fat around the abdominal viscera, and is associated with dyslipidemia (increased plasma triglyceride, low high-density lipoprotein cholesterol) and poses a greater risk of diabetes mellitus,

insulin resistance, hypertension, metabolic syndrome, and cardiovascular disease than does peripheral obesity.(Busetto, 2001). Surgical removal of visceral fat leads to the improvement of insulin resistance in rodents (Barzilai et al. 1999).

Obese subjects have high levels of ectopic intra-muscular fat deposition which correlates positively with visceral fat levels. (Hansen et al, 1997). It has been shown that intra-muscular fat deposition correlates strongly with whole body insulin resistance (Soo et al, 2008, Tisha and Steven, 2007), possibly by reducing skeletal muscle insulin receptor signalling (Melpomeni et al, 2007).Furthermore, a moderate reduction in the triglyceride content of muscle via weight loss was shown to improve insulin resistance.(Mazzali et al, 2006)

2.1.9 The challenge of treating diabetes

Although the modern allopathic system of medicine is greatly accepted in the treatment of diabetes throughout the world, managing both Type 1 and Type 2 diabetes remains a major challenge. The present mainstream drugs for treating the disease, though effective have a number of limitations and challenges to the administration of health care in diabetic patients, including side effects and primary and secondary drug failures (Kochhar and Malkit, 2005).

Patients are known to experience both primary failure, where the patient does not respond to the initial medication for treatment and secondary drug failure where failure in treatment sets in after an initial response to the treatment. Secondary failure to oral hypoglycemic agents is defined as a good initial response to oral agents (at least one month) with decreasing effectiveness and eventual failure. In such cases, the drug therapy is changed or extra therapy is added. Ultimately, most Type 2 diabetic patients will need exogenous insulin therapy (Knut, 1999). The incidence of secondary drug failure, as published in literature is 0.3% to 30% (Batra, 1991).

In Type 2 diabetes, the successive failure of non-pharmacologic therapy and oral antihyperglycemic agents eventually burdens patients with a heavy history of uncontrolled hyperglycemia (Brown et al, 2004, Maru et al, 2005).

The use of anti-diabetic drugs is a continuous process, and patients have to take these drugs daily. One alternative that has shown some promise in helping overcome some of these challenges are medicinal plants used individually or in

combination. Among the many diseases that have led to the increased use of medicinal plants is diabetes mellitus**.** Several herbs have been shown to be highly effective in controlling blood sugar concentration and reducing problems associated with diabetes. These herbs sometimes may act as a monotherapy and most of the time they can be taken in addition to other anti-diabetic drugs (Eddouks et al, 2002, Omar et al, 2008). It is interesting to note that one of the most common drugs used for the treatment of Type 2 diabetes is metformin, the parent drug having been originally derived from the French lilac (Witters, 2001).

2.2.0 Anti-diabetic drugs used for managing insulin resistance

The challenges and economic threats posed by the disease highlighted above has necessitated continual research into potential new therapies for the treatment of diabetes and insulin resistance. Even though no known cure for diabetes has been discovered, a number of medications have been used to treat and manage the ailment successfully.

Given the association between type 2 diabetes and insulin resistance, most of the classes of drugs used for the treatment of diabetes are potent insulin sensitising agents. These classes of drugs include the biguanides and the thiazolidinediones.

Examples of thiazolidinediones include rosiglitazone and pioglitazone. While metformin is the most widely used biguanide. Thiazolidinediones and biguanides counter insulin resistance, but act by different mechanisms. The two agents are able to lower blood

glucose concentrations in type 2 diabetes without occurring overt hypoglycemia and both require the presence of insulin to generate their therapeutic effects, but act without stimulating insulin secretion (Masuda and Terauchi, 2010).

The two agents are also used in combination therapy to manage diabetes and insulin resistance. Metformin exerts a stronger suppression of hepatic glucose output, while thiazolidinediones produces a greater increase in peripheral glucose uptake, enabling metformin-thiazolidinedione combinations to improve glycaemic control in type 2 diabetes with additive efficacy. Basal insulin concentrations are not raised by metformin or thiazolidinediones, so there is minimal risk of hypoglycaemia, and metformin can reduce the weight gain associated with thiazolidinediones (Bailey, 2005)

Single pill combinations of rosiglitazone/metformin and pioglitazone/metformin have recently been approved for use in the US and Europe. (Masuda and Terauchi, 2010)

Metformin has been shown to act via activating the intracellular kinase, AMP-activated protein kinase (AMPK) (Sirtori and Pasik, 1994). This enzyme is able to increase cellular glucose uptake and reduce hepatic gluconeogenesis. The thiazolidinediones act via binding to the transcription factor peroxisome proliferator-activated receptor (PPAR)-□, which is highly expressed in adipocytes. This leads to increased adipogenesis and the production of small, insulin sensitive adipocytes, which may act as glucose “sinks” (Lessard et al, 2007). Activation of PPAR-□ also leads to increased adipocyte secretion of adiponectin and reduced secretion of TNF□ (Hannele, 2004).

2.2 Traditional medicine

Traditional medicine (TM) is the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness. It also refers to health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well-being. (World Health Organization, Ernst et al, 1995).

2.2.1 Increasing use and popularity of traditional medicine

Traditional medicine has maintained its popularity in all regions of the developing world and its use is rapidly spreading in industrialized countries.

- In China, traditional herbal preparations account for 30%-50% of the total medicinal consumption. (WHO, Fact sheet N°134, 2003).
- In Ghana, Mali, Nigeria and Zambia, the first line of treatment for 60% of children with high fever resulting from malaria is the use of herbal medicines at home. (WHO, Fact sheet N°134, 2003).
- World Health Organization estimates that in several African countries traditional birth attendants assist in the majority of births. (WHO, Fact sheet N°134, 2003).

- In Europe, North America and other industrialized regions, over 50% of the population have used complementary or alternative medicine at least once. (WHO, Fact sheet N°134, 2003).
- In San Francisco, London and South Africa, 75% of people living with HIV/AIDS use TM.
- 70% of the population in Canada has used complementary medicine at least once. (WHO, Fact sheet N°134, 2003).
- In Germany, 90% of the population has used a natural remedy at some point in their life. Between 1995 and 2000, the number of doctors who had undergone special training in natural remedy medicine had almost doubled to 10 800. (WHO, Fact sheet N°134, 2003).
- In the United States, 158 million of the adult population use complementary medicines and according to the USA Commission for Alternative and Complementary medicines, US $17 billion was spent on traditional remedies in 2000. (WHO, Fact sheet N°134, 2003).
- In the United Kingdom, annual expenditure on alternative medicine is US$ 230 million. (WHO, Fact sheet N°134, 2003).
- The global market for herbal medicines currently stands at over US $ 60 billion annually and is growing steadily (WHO, Fact sheet N°134, 2003).

2.2.2 Tried and tested methods and products

It has been estimated that 25% of modern medicines are made from plants first used traditionally (W.H.O.). Traditional medicine can have an impact on infectious diseases. For example, the Chinese herbal remedy *Artemisia annua*, used in China for almost 2000 years has been found to be effective against resistant malaria and could create a breakthrough in preventing almost one million deaths annually, most of them children, from severe malaria (WHO monograph on good agricultural and collection practices (GACP) for *Artemisia annua* L.)

In South Africa, the Medical Research Council is conducting studies on the efficacy of the plant *Sutherlandia Microphylla* in treating AIDS patients. Traditionally used as a tonic, this plant may increase energy, appetite and body mass in people living with HIV (WHO, Fact sheet N°134,2003).

It is known that about seventy countries have a national regulation on herbal medicines but the legislative control of medicinal plants has not evolved around a structured model. This is because medicinal products or herbs are defined differently in different countries and diverse approaches have been adopted with regard to licensing, dispensing, manufacturing and trading (WHO, Fact sheet N°134, 2003).

2.2.3 Traditional medicine use in Africa

The use of traditional herbal medicine on the African continent has continually been on the rise due to the following reasons:

- It is cheaper than orthodox medicine in Africa, more accessible to the African villagers and is more acceptable to Africans than modern medicine.
- Alternative and traditional medicine (ATM) is a potential source of new plant derived drugs, a source of cheap starting products for synthesis of known drugs, or a cheap source of known drugs such as reserpine, vinblastine, etc.
- Alternative and traditional medicine remedies are mostly compounded from natural products (herbs); so they should be readily acceptable to the body. (Sofowora, 1996)**.**
- Traditional medicinal plants are presently used by over 70% of the world's population according to the World Health Organization. This trend has continued to experience increased growth in patronage as they are considered to be safer with less toxicity, readily available, more affordable and accessible particularly to the indigenous populations of third world nations where medical plants remain the main form of health care treatment (Website reference 5).

2.3 Medicinal plants in diabetes research and treatment.

Plants remain a major source of raw materials for medicines and drugs. Research is on going to discover novel anti-diabetic drug candidates from plants. Till date, about 400 plant species have been identified and documented as medicinal plants with anti-diabetic properties (Ajay et al, 2006).

Presently, several research projects on anti-diabetic medicinal plants are at different stages of development. A good example is dianex, a poly herbal formulation consisting of the aqueous extracts of *Gymnema sylvestrte, Eugenia jambolana, Mormordica charantia, Azadirachta indica, Cassia auriculata, Aegle marmelose, Withania somnifera* and*Curcuma longa,*which was shown to produce significant hypoglycemic activity in both normal and diabetic mice while also reversing other diabetic complications. (Mutalik et al, 2005).

Jia et al (2003) have documented the results of their research on seven commercially available Chinese polyherbal products with each polyherbal formulation producing significant dose-dependent blood glucose lowering activity in various animal models as well as in humans.

Korean scientists working on a polyherbal formulation “SMK001” concluded that continuous treatment of rats made diabetic by the use of stretozotocin with SMK001 produced a significant decrease in the blood and urine glucose concentration (Jong et al, 2006).

Also, household plants like ginger and garlic have been widely reported to positively impact and improve the state of diabetic subjects (Khaled et al, 2008).

2.4 About the plants used in this study

2.4.1 Icacina trichantha *(Family,Icacinaceae)*

The plant is a shrub that arises from a stout hairy underground tube with a stem straggling with soft brown hairs. The flowers are cream, petals hairy with slightly hairy fruits up to 2.5 cm when ripe. This undergrowth is common in West Africa and Southern Nigeria.(Gbile et al, June 1993).

Acute toxicity testing of aqueous extracts of the tuber in mice at a maximum administered dose of 1.6g/kg body weight resulted in 40% death. No death was observed at 800mg/kg body weight administered to the animals (Asuzu and Ugwueze, 1990). The tuber of *icacina trichantha* extracted with 50% methanol induced sleep in rats treated with doses ranging from 400 -1000 mg/kg body weight intraperitoneally (i. p). The LD_{50} of the methanolic tuber extract in rats was 671mg/kg body weight, i p (Asuzu and Abubarkar, 1995). Moderate antioxidant property was reported by Oke and Hamburger, (2002). No analysis of the anti-diabetic properties of this plant has been conducted despite its traditional use in the treatment of diabetes.

2.4.2 Uraria picta *(Jacq.) DC. (Family,Leguminosae-papilionoideae)*

The plant is an herbaceous annual plant growing to between 0.5 – 1 meter in height. The stems are covered with short crooked hairs. Fruits are made of short pods with 4

to 5 articles twisted like an accordion. The plant is a paleotropical species, growing in all the African savannahs, south of the Sahara (Adjanohun et al, 1991).

Ethno-medical uses of the plant, *Uraria picta*, in Nigeria include the treatment of skin diseases and for accelerating fracture wound healing. The plant extract is also used to control ecto-parasites in man and domestic animals. Methanolic and aqueous LC_{50} of the plant were reported as 2,700 micrograms/ml and 57,000 micrograms/ml in ticks (Igboechi and Osazuwa1988).

The combination of the plant species, *icacina trichantha, ananas cosmos and uraria picta* are also used for the treatment of diabetes. There are no publications on the anti-diabetic effects of the plants. This study is being undertaken to investigate the traditional evidence of the blood sugar lowering properties and the use of *icacina trichantha, ananas cosmos and uraria picta* in the treatment of diabetes.

2.4.3 *Ananas cosmos* *(Family,* Bromeliaceae)

The plant belongs to the same family as the popular pineapple *Ananas comosus* (family Bromeliaceae) and in most documentations, they are ascribed the same botanical name. The two plants are however different in size, with the normal pineapple being bigger in size and sweeter in taste. For the purpose of the present studies, the plant has been identified as *Ananas cosmos* with a preserved voucher specimen. The plant is grown in different parts of the world.

The formulation for the above named plants was obtained from a Nigerian herbalist (Mr. Adeyemi, 19 Igbobi college road, Shomolu, Lagos) who had used them extensively for a number of years to treat and manage diabetes with positive blood sugar control results. In one particular elderly patient, her personal physician after examining and taking her blood sugar level was amazed at the results. On further questioning, his patient confirmed to him that she was on herbal medication. The doctor advised her to continue with the treatment as it gave her more relief than the allopathic prescriptions.

There is no formal documentation of these plants or the combination of the three plants being used to treat or manage diabetes mellitus therefore, it needs to be investigated more formally.

CHAPTER 3 MATERIALS AND METHODS

3.1 Preparation of plant material

The plants used in this study *Icacina tracantha (*tuber*) (fam. Icacinaceae), Ananas cosmos* (fruit) (*fam. Bromeliaceae), and Uraria picta (leaves) (fam. Papilionaceace),* were procured from a market in Mushin, a suburb in Lagos, Western Nigeria.

The plants where not randomly procured. The procedure for procuring the plants from Mushin market was undertaken to simulate the procedure of the traditional herbalist, who regularly buys plants from the same seller who in turn obtains the plants from villages in a particular geographical area. Advice was sought from the traditional healers in terms of which sellers have the most efficacious plants.

The plants were identified at the Institute for Tropical Agriculture, Ibadan, Oyo State, Nigeria and voucher specimens were deposited for reference and preservation at the institute's herbarium. The three herbal medicinal plants were studied individually and in combination for their effects in a rat animal model of insulin resistance.

3.1.1 Equipment used for extraction of aqueous crude extracts from the plants

- Mettler PM4600 weighing scale.Mettler-Toledo AutoChem Inc., 7075 Samuel Morse Drive, ColumbiaMD, 21046USA

- 250ml glass beakers Boeco, Germany

- Drying oven, Memmert Schwabach, Germany

- Standard laboratory Bunsen burner,

- Brannan Mercury thermometer. S. Brannan & Sons Ltd, England

- 3000ml Pyrex beakers, Pyrex.

- Labcon low temperature incubator. Labmark Laboratory Marketing Services (Pty) Ltd, Roodepoort, Johannesburg, South Africa.

3.1.2 Procedure for preparation of crude plant extracts

A 1.25 kg batch of dried macerated individual herbs was weighed using a Mettler PM4600 balance, washed in 5 litres of distilled water and the water was then removed by filtering the cleaned plant material through a sieveof aperture size, 710µm.The clean plant was placed in a 3 litre beaker. Distilled water was added to cover the plant in the beaker. The beaker containing the plant and distilled water was then heated and allowed to boil at a temperature of 100^{o}C for 2 hours. Distilled water was added intermittently to ensure the plant material was always covered. The preparation was allowed to stand at ambient temperature and cool for about 12 hours. It was then filtered with a sieve of aperture size, 710µmand the filtrate containing the plant extract was poured into a number of pre – weighed 250ml

beakers (Boeco, Germany) and allowed to dry in the oven at a temperature of 50^{o}C until the filtrate was evaporated to dryness leaving the crude plant extract.

The quantity of the plant extract obtained from the plant was determined by calculating the difference between the weight of the pre – weighed beakers and the weights of the beakers containing the dried plant extract. The dried extract was stored at a temperature of 4^{o}C in a Labcon low temperature incubator for appropriate use when the doses were formulated and administered to the animals as required. The extraction ratio was determined using the weight of the extract as a percentage of the original dried plants. This method of extraction was adopted to simulate the traditional method of extracting the plants. This procedure was carried out in obtaining crude extracts for all the plants used in this study.

To obtain a mixture of the plant extracts, the plants were combined based on the ratio used for treating diabetic patients as used in the traditional herbal clinic in Nigeria. Thus, 3 parts of *Uraria picta* to 2 parts *Ananas cosmos* to 1 part of *Icacina tracantha* was weighed, combined and extracted as described previously.

3.2 Toxicity studies.

Toxicity studies were conducted to ascertain the safe doses of the plant extracts to be administered to the rats in this study. This was necessary because previous work on the plants has only been able to identify the acute toxicity doses of some of the plants (Igboechi and Osazuwa, 1988, 1989; Asuzu and Abubarkar, 1995; Asuzu and

Ugwueze, 1990) whereas the present study involved the chronic administration of the plant extracts for a period of three weeks.

Two toxicity studies were conducted. The first study was performed using doses of *IIcacina tricantha* of 100mg/kg, *Ananas cosmos* of 200mg/kg, and *Uraria picta* of 300mg/kg body weight. These doses were chosen based on published acute toxicity studies (Igboechi and Osazuwa, 1988, 1989; Asuzu and Abubarkar, 1995; Asuzu and Ugwueze, 1990).

When the plant extracts were actually tested for their ability to improve glucose tolerance it was found that the *Ananas cosmos* and *Uraria picta* both lowered glucose concentration during the OGTT in the rats receiving the normal rat chow diet but had no effect on the animals receiving the high calorie diet. The *IIcacina tricantha* had no effect in either group of animals. It was therefore decided to give the high calorie diet-fed animals higher doses of *Ananas cosmos* and *Uraria picta*. Therefore, a second toxicity study had to be performed to ensure that these higher doses (600 mg/kg of AC and 800 mg/kg of *Uraria picta*) were safe.

For the first toxicity study, nine male Sprague-Dawley rats weighing approximately 300 grams were used. The animals were divided into groups of three animals per group.The maximum dose of the extracts (100mg/kg for IT, 200mg/kg for *Ananas cosmos*, and 300mg/kg body weight for *Uraria picta*) to be administered per kilogram body weight in the main study was administered to the animals daily for three weeks.

For the second toxicity studies using higher doses of *Ananas cosmos* and *Uraria picta*, four animals were used to reduce the suffering and death on the animals as a first toxicity test had been performed earlier. The animals were divided into two groups of two animals each, with each group being allocated to each plant extract. The animals were then given the allocated doses of the different plant extracts orally for 3 weeks at doses to be used in the main experiment. All the animals were dosed daily for the period of 3 weeks. During this period, the animals were observed for symptoms, rate of movement, general activity, behavioural changes and death.

3.3 Preparation of rats for extract dosing

Male Sprague-Dawley (SD) rats weighing between 200 – 240 grams (217.45 ± 13.57) were used for the investigation of the possible anti-diabetic activity of*Icacina tricantha*, *Uraria picta* and *Ananas cosmos*.

Two to three rats were housed in each cage (n=2 or 3). All cages were numbered for proper identification, while all animals were marked with water resistant makers on their tails. The marking of the tail was done weekly to ensure that the animals could be properly identified.

After 1 week of acclimatization to the facility, feeding of the animals commenced. The first batch of animals (n=54) was made up of the control animals fed on normal rat chow. All animals were weighed before the commencement of feeding. Animals were then allowed free access to food and water for the next four months. All animals were weighed monthly. This same procedure was applied to the animals given the high calorie diet.

3.3.1 Recipe for the special diet

The second experimental batch of animals (n=66) was fed on a special high calorie diet. The diet consisted of 1.32kg of normal rat chow, 812ml of distilled water, 280 grams of brown sugar (Selati® golden brown sugar) and 4 cans of normal condensed milk (Clover® condensed milk, 370 grams) that was mixed together to produce a homogenous paste. The paste was fed to the animals daily in a bowl. All rats in this

experimental group were placed on the diet for four months. This formulation was made every three days and the remnant discarded to avoid the growth of fungus.

Food description	Calorie (cal)
Carbohydrate from sugar	6591
Carbohydrate other from plant sources	3595
Protein	2032
Fats	2835

Table 3.0: Estimated nutritional value of the high calorie diet.

At the end of the feeding period of four months, all the animals were randomly divided into groups containing six animals (n=6) per group as shown in table 3.1. For the next three weeks of the dosing of the rats with the various doses of the plant extracts, food consumption was measured and calculated. It was done to also determine the effect of the plant extract on the quantity of food consumed by the animals. For the animals fed on the normal rat chow, the feed in the cages was weighed before it was given to the animals and then the remnant weighed every three days. Enough food was left to last beyond the 3 day period. The difference in weight between the initial quantity of food and the remnant was calculated and was taken as the food consumed by the animals in the cage. After taking measurement of the food consumed, fresh food was weighed to replenish the consumed food. This figure was divided by the number of animals per cage and by the number of days to estimate the quantity of food consumed by each animal per day it is assumed that each rat consumes roughly the

same amount of food. For the animals on the special diet, the food consumed was measured by first weighing the bowl and then weighing the bowl with the feed paste. After the three day period when the feed was changed and freshly prepared feed introduced, the bowl and the remnant feed was weighed and the difference was divided by the number of animals per cage and by the number of days to give the average feed consumed by the animals per day.

Table 3.1: Group of rats and the daily dosing regimen administered.

Doses of plant extracts administered	*number of* animals per group
Group A: Normal rat chow group:	
Icacina tracantha	
50 mg/kg body weight	6
100 mg/kg body weight	6
Uraria picta	
150 mg/kg body weight	6
300 mg/kg body weight	6
Ananas cosmos	
100mg/kg body weight	6
200mg/kg body weight	6
Ananas cosmos/Icacina tracantha/Uraria picta (300 mg/kg) combination	6
Reference groups	
Metformin (Sigma - Aldrich) 300mg/kg	6
Control group (on water only)	6
Sub Total	54

Group B: High calorie diet group.	*no of* animals per group
Icacina tracantha	
50 mg/kg body weight	6
100 mg/kg body weight	6
Uraria picta	
150 mg/kg body weight	6
300 mg/kg body weight	6
800mg/kg body weight	6
Ananas cosmos	
100mg/kg body weight	6
200mg/kg body weight	6
600 mg/kg body weight	6
Ananas cosmos/Icacina triacantha/Uraria picta (300 mg/kg) combination	6
Reference Groups	
Metformin group 300mg/kg daily	6
Special diet control group (on special diet only)	6
Sub Total	66
Total number of animals used	**120 rats.**

3.4 Weighing of animals

After feeding for a period of four months, dosing commenced. During the period of dosing, all animals were weighed on a weekly basis using a Mettler PM4600 electronic scale.

3.5 Dosing animals

After the animals were weighed, doses of the plant extract to be administered were calculated and formulated per body weight for each of the animals. This was done by dissolving the plant extract in distilled water, making the appropriate concentration and determining the dose to be administered. The animals were then each given the calculated dose of the plant extractdaily between 7 – 10 am in the mornings by oral gavage with the aid of a dosing needle and syringe for 3 weeks. The doses given of each plant extract are shown in Table 3.1.

Before the commencement of dosing with the different plant extracts, 1ml of blood was collected from the cleaned tail of each animal. This was necessary to have a baseline reading for the plasma blood samples from each animal. Weekly, 1 millilitre of blood was collected from the tail vein of all the animals with the help of the veterinary nurses and animal technicians of the central animal unit.

3.6 Blood collection

Prior to the collection of blood, the rats were restrained in a restraining device and the tail cleaned with an alcohol swab (see Figure 3.6a). A 1 ml syringe was used to collect blood from the tail vein of each rat. All syringes and 1.5 ml Eppendorff tubes for collecting blood were flushed with the anticoagulation agent, heparin. Blood was collected before the dosing of the animals, after week one, week two and at termination.

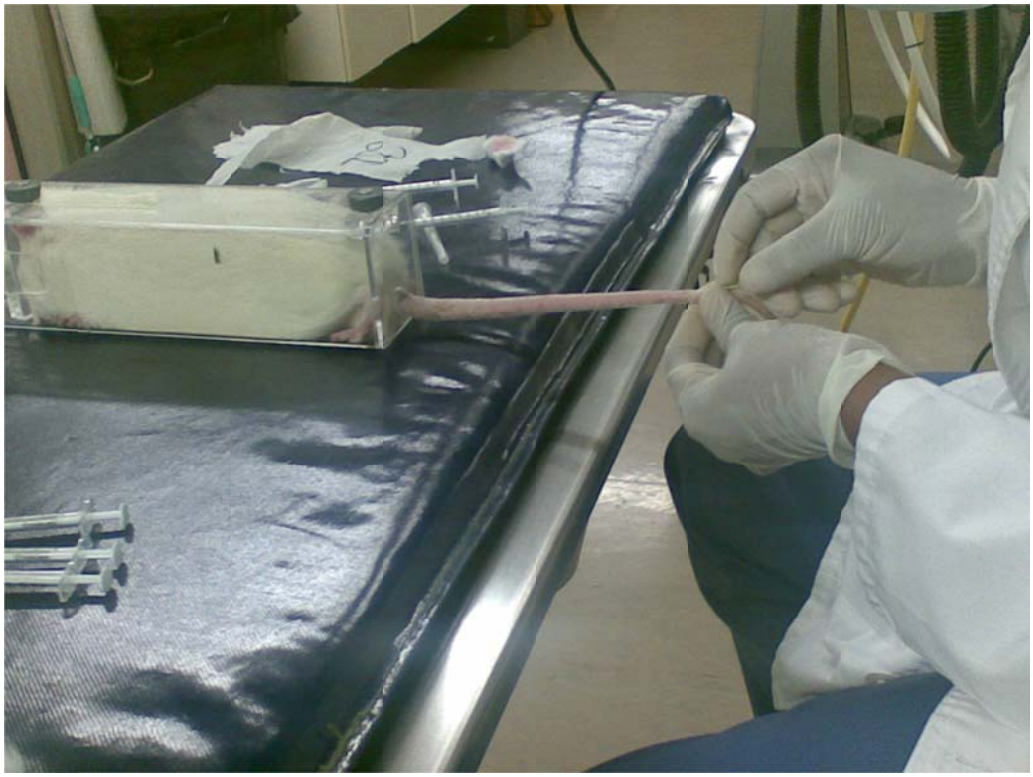

Figure 3.6a: Rat in restraining device during the collection of blood.

The collected blood was kept in the 1.5 ml Eppendorf tubes and immediately stored at a temperature of between 0 - 1 ^{0} C by placing the tubes in ice boxes containing ice.

This temperature was maintained with the aid of a thermometer and constantly adding more ice. This was done to inhibit the breakdown of the glucose in the blood as no anti - glycolytic agent was used at this stage (Annelise et al, 1998).

The blood in the Eppendorf tubes were centrifuged at 3000g using a Sorvall T6000D centrifuge and plasma collected by carefully extracting with a pipette. The collected plasma was stored in sterile 1.5ml Eppendorf tubes at a temperature of -72^{0} C in a Sanyo Ultra low temperature freezer (Model - U4086S) until further analysis.

3.7 Oral glucose tolerance test

3.7.1 Material and equipment

Accu-check active glucometer (Roche Diagnostics, Basel, Switzerland)

Accu-check active glucometer strips (Roche Diagnostics, Basel, Switzerland)

Glucose obtained fromSaarchem chemicals, South Africa.

Dosing needle and 5 ml syringes (SURGI PLUS), swab, and distilled water.

250 millilitre beakers, Pyrex, 200 millilitre flask Schott Duran

Balances: Mettler – Toledo AB104 – S, Mettler PM4600.

3.7.2 Experimental procedure for measurement of glucose concentration

After the daily dosing period of three weeks and weekly collection of blood, an oral glucose tolerance test (OGTT) was carried out on all the animals. All rats were fasted overnight and the following morning2g/kg body weight of glucose solution was administered to each animal by oral gavage.

Increased glucose concentrations have been associated with diabetes mellitus. An OGTT is used to determine the ability of the body to metabolize and clear glucose out of the blood stream. The test can be used to determine and diagnose diabetes. Administering the test after the period of dosing will help determine the effect of the plant extract on glucose clearance. Where the plant extracts have the ability to

improve glucose clearance rate, it is expected that the area under the curve for the plant extract will be lower compared to the control.

Blood glucose concentrations were measured by pricking the tail tip and using an *Accu-check active (Roche Diagnostics, Basel, Switzerland)* blood glucose meter to take the readings. The glucose level was read by allowing a drop of blood from each animal's tail to drip on the glucometer strip (Accu-*Chek* active strips, *Roche*) inserted into the glucometer. The glucose concentration was then read and documented from the glucometer readings. This method was chosen because it used a very small quantity of whole blood (<5 µl) and gave immediate results and thus an indication of whether the glucose load had been administered successfully via gavage. Results were not repeated due to the high cost of the Acu-Check strips and ethical considerations of not putting rats under more stress than what is required. After the initial measurement of blood glucose was taken using the glucometer (at time 0), glucose was administered orally to each rat. Blood glucose concentrations were then measured at 30, 60, and 120 minutes after the loading dose of 2g/kg glucose was given.

One day after the completion of the OGTT, all animals were anesthetized, 5ml of blood was collected using cardiac puncture into grey BD Vacutainer bottles and portions of the liver, left soleus and gastronimus muscles, pancreas, and abdominal fat tissues were collected into 1.8 ml cryogenic tube vials, (Nunc, Denmark). This was done to collect final blood samples and relevant tissues (liver, muscles and fat) that

will further help to shed more light on the effect of the plant extracts on glucose, insulin and free fatty acid in the body.

3.8 Euthanizing of animals and harvesting of tissues

This procedure was performed under the guidance of the staff of the University of the Witwatersrand animal house.

3.8.1 Anesthetizing and euthanizing procedure

A mixture of ketamine (80mg/kg body weight) and xylazine (40mg/kg body weight) was used to euthanize the animals. The anaesthetic was administered intramuscularly.

3.8.2 Collection of final blood samples and harvesting tissues

After the animals were anesthetized, 4mls of blood was collected via cardiac puncture with a needle and a 5 ml syringe into grey BD Vacutainer bottles. Each tube was gently shaken to ensure proper mixing of the blood with the anti-coagulant before centrifuging.

Using a surgical blade, a pair of scissors and forceps, each animal was dissected and portions of liver, left soleus and gastronimus muscles, pancreas, and abdominal fat tissues were collected into 1.8 ml cryogenic tube vials, (Nunc, Denmark) and quickly snap frozen in liquid nitrogen in a Dewar flask and stored at – 72 ^{0} C in a Sanyo Ultra low temperature freezer, (Model - U4086S) for further analysis.

The tissues were identified with the help of the consultant veterinarians at the University of the Witwatersrand Central Animal Unit. The collected blood was collected into grey BD Vacutainers and processed as mentioned in section 3.6.

3.9 Analysis of plasma samples for glucose concentration using the Quantichrom glucose assay kit

The procedure was carried out using the Quantichrom glucose assay kit using 96-well plates. The kit was procured from BiosAssay Systems, Hayward, United States. Catalogue number DIGL-100, DIGL-200. This method was chosen to measure glucose concentration during the period when the animals were being treated with the plant extracts.

In the diabetic state, the glucose concentration in the body is elevated and needs to be normalized. While the OGTT is performed to determine the clearance rate of glucose from the blood, the analysis of the plasma for glucose concentration helps to determine the effect of the plant extracts during the three week dosing period on the mobilization of glucose from the blood into the storage organs, the muscles and the liver. It is expected that plant extracts that have anti-diabetic properties will show a reduced concentration of glucose in the blood plasma compared to the control, indicating that the plant extract have a greater ability to mobilize glucose compared to the control group. The lower this concentration, the more efficacious the plant is in performing this function. Thus, this test is needed to help investigate the diabetic action of the plants.

3.9.1 Material and equipment used

Labsystem finnipettepipette. Labsystem

Multiple pipette dispenser (Eppendorf multipetter plus).

Disposable tips.

1.5 and 2.0mL Eppendorf centrifuge tubes

Orbital plate shaker.

Tube holder.

Labcon water bath.

-20 ^{o} C freezer

96 well plate, Sero-Wel, Bibby Sterillin Ltd, U.K.

Labsystems Multiskan Ascent automatic plate reader. (Labsystem Inc. United States)

3.9.2 Experimental procedure

The 1.5mL Eppendorf centrifuge tubes were set up in a tube holder. Following this 5 µL diluted standard glucose solution from the manufacturer's kit for the standard curve, and plasma samples from each rat, were transferred into appropriately labeledEppendorf tubes using the mutipipette dispenser and disposable tips. This was followed by the transfer of 500 µL of reaction reagent (1)from the glucose assay kit into each tube using a multiple pipette dispenser (Eppendorf multipetter plus).

The tubes were tightly closed and thoroughly mixed by shaking andthen placed in the tube holder and heated in a boiling water bath (Labcon) for 8 min. After 8 min, the tubes were cooled down in a cold water bath for 4 min at 4 ^{0} C. Then, 200 µL of the

contents of each of the tubes were transferred using a pipette (Labsystem finnipette pipette) with attached tip into each well in a clear bottomed 96-well plate, while avoiding the formation of bubbles. The plate was read at a wavelength of 650nm. Actual glucose concentration in the individual rat plasma samples was calculated from the optical density readings by using equation 3.1 below. The results were converted to mmol/L.

The optical density (OD) of the water blank was subtracted from the standard OD values and these were plotted against the known standard concentrations that came with the kit. The slope was determined using linear regression fitting. Because the standard curve went through the origin, the glucose concentration in each plasma sample is calculated as in equation 3.1.

$$\text{Glucose concentration} = \frac{OD_{SAMPLE} - OD_{BLANK}\ (mg/dL)}{\text{Slope}} \qquad \text{Equation 3.1}$$

Where OD_{SAMPLE} and OD_{BLANK} are optical density values of the plasma samples and "blank" samples respectively.

3.10 Analysis of insulin concentration in rat plasma samples

Insulin the hormone responsible for the mobilization of glucose from the blood is necessary to help maintain normal glucose concentration. In the present experiment, the animals have been fed a high calorie diet that leads to high concentration of insulin and insulin resistance. If the concentration of insulin is not normalized overtime, type II diabetes sets in. Substances that help bring down the concentration

of insulin and improve the mobilization of glucose into the cells may be described as having anti-diabetic effect. In the present experiment, lower concentration of insulin over time by any of the plant extracts in comparison to the control is an indication that the plant extracts have glucose lowering effects

3.10.1 Material and equipment used

High Range Rat Insulin ELISA, catalogue number 10-1145-01, Mercodia, AB,

Labsystem finnipette pipette.(Labsystem Inc. United States).

Multiple pipette dispenser (Eppendorf multipetter plus).Uppsala, Sweden.

Disposable tips.

Orbital shaker and incubator for plates.

BioTek ELX 50 automated strip washer.

Labsystems Multiskan Ascent automatic plate reader.(Labsystem Inc. United States)

3.10.2 Experimental procedure

The concentration of insulin in the rat plasma samples for all the animals was analyzed by using High Range Rat Insulin ELISA, catalogue number 10-1137-01 procured from Mercodia, AB, Uppsala, Sweden. A 5 µl sample of plasma from each ratwas introduced into each well of a 96 well plate, using a Labsystems finnipette pipette with a disposable tip.

A 5 µl aliquot of each calibrator was also added to the appropriate wells in the 96 well plate. This was followed by the addition of 50 µl enzyme conjugate solution to all the wells.

The plate was incubated on a plate shaker at 700 – 900 rpm for 2 hours at a temperature of 24^0 C. The 96 well plates was then washed using a BioTek ELX 50 automated strip washer, six times. After the final wash, the plate was blotted on a clean tissue to ensure that the plate was dry and then200 µl of the substrate, TMB was added to each well. The plate was further Incubated for 15 minutes and50 µl of stop solution was then added and the plate was put on the shaker for about 5 seconds to ensure thorough mixing of the contents. After mixing, each plate was read using a Labsystems Multiskan Ascent Spectrophotometer plate reader at 450nm. The actual insulin concentration in the plasma samples were calculated from the spectrophotometer readings using the standard curve determined from the supplied calibrators. The results were recorded as µU/mL.

3.10.3 Calculation of Insulin sensitivity

Insulin sensitivity in each animal was calculated using the homeostasis model assessment (HOMA-IR) formula:

Fasting insulin (µU/mL) × fasting glucose (mmol/L)/22.5.Equation 3.2

Low HOMA-IR values indicate greater insulin sensitivity, and higher HOMAR-IR values indicate lower insulin sensitivity (insulin resistance).

3.11 Analysis of free fatty acid concentration in rat plasma samples

Because obesity and dyslipedemia are associated with diabetes, the ability of the plant extracts to improve the lipid profile by reducing the concentration of free fatty

acid in comparison to the control group is an indication of the anti-diabetic properties of the medicinal plants.

3.11.1 Equipment and reagents.

Roche kit: free fatty acids, half – micro test, Cat N^{o} 1383175

Table 3.11.1a Content of kits

Bottle	Contents
1	5 x 11 ml each of potassium phosphate buffer, pH 7.8
2	*5 tablets,each tablet contains: ATP, coenzyme A, acyl-CoA-synthetase (Acyl CS), peroxidase, ascorbate oxidase, 4-aminoantipyrine and stabilizers.*
3	3 mlof aqueous N-ethyl-maleinimide solution with stabilizers. *Note:* The presence of N-ethyl-maleinimide in the test is necessary for the removal of an existing surplus of CoA before the oxidation of the activated fatty acids by ACOD.
4	5 x approx. 0.6 ml of ACOD dilution solution and stabilizers.
5	5 tabletsand each tablet contains: acyl-CoA-oxidase (ACOD) and stabilizers

96 – microwell plate Sero – Well (Bibby Sterilin Ltd, Stone Staffs, U K)

96 well plate, Sero-Wel, Bibby Sterillin Ltd, stones U. K.

Eppendorf multipettor

Accurate pipettes for 10ul volumes

Orbital shaker and incubator for plates.

Labsystems Multiskan Ascent automatic plate reader

3.11.2 Procedure

The manufacturer's protocol was followed and used in this procedure. All the solutions and reagents were mixed and prepared as described in the kit.Using a pair

of forceps to transfer the tablets from the bottles, reaction mixture A was prepared by dissolving one tablet from bottle 2 in the solution in bottle 1. Reaction mixture B was prepared by dissolving one tablet of bottle 5 in the solution in bottle 4. The prepared solutions were stored at 4^{o} C in a Labcon low temperature incubator. This was used up within 3 days as the prepared solutions do not remain stable for long.

A standard solution of palmitate, was prepared as documented in the manufacturer's kit.Thus, working solution 1 was made by dissolving 6.0 g of Triton X-100 (BDH. Poole, England) in about 80 ml of warm double distilled water in a 200ml beaker (30°-40°C), allowed to cool to 15-25°C and made up to 100 ml in a measuring cylinder. Next, 9 mg palmitic acid was weighed using a Mettler – Toledo AB104 – S balance, introduced into a 100 ml beaker and dissolved in about 6 ml of warm ethanol (about 35 – 40 °C). The beaker was immediately sealed with parafilm and allowed to cool to 15 – 25 °C to make working solution 2.

The two working solutions were combined to create the standard palmitc acid solution which is stable for only 3 days in a refrigerator. A 10 µl aliquot of blank (double distilled water), standard and samples were distributed into the wells of a 96 well plate using an Eppendorf pipette with disposable tips. Next, 200µlof solution A was added into each well using an Eppendorf multipettor plus.
The plate was then placed on the orbital plate shaker and incubator (around 500 – 800 rpm), and incubated at a temperature of 25 ^{0} C for 10 minutes. After incubation, 10 µl of N-ethyl-maleinimide stop solution was added into all the wells to stop the

reaction. The plate was then mixed shortly on the shaker and absorbance read at 540 nm using a plate reader (Labsystems Multiskan Ascent), (A_1). The plate was returned to the shaker for 20 minutes after which readings were taken again using the plate reader (A_2). The difference in absorbance readings (A_2 - A_1) for all the samples, including both blank and sample was calculated. The absorbance difference of the blank (ΔAb) was then subtracted from the absorbance difference of the sample (ΔAS). This gives (ΔA)the concentration of free fatty acid in each plasma sample as shown by equation 3.3.

$\Delta A = \Delta AS - \Delta Ab$ Equation 3.3

3.12 Statistical analyses

Area-under-the-curve (AUC) values for glucose during the OGTT were calculated using the trapezoid rule. Fasting and AUC glucose concentrations were compared across groups using 1-way ANOVA ..

At baseline (i.e. before administration of the plant extracts), significant differences were observed between the rat groups for the mean values of nearly all the variables under analysis. Thus, for the normal diet fed animals the minimum and maximum mean baseline values for body weight, glucose, free fatty acids, insulin and HOMA for each of the 9 treatment groups were as follows: 600-697 g; 5.1-7.2 mmol/l; 0.61-1.23 mmol/l; 3.86-6.32 μIU/ml; 0.83-1.87. For each of these variables, except insulin, ANOVA demonstrated a statistically significant difference across the 9 groups of animals. For the high calorie diet fed animals the minimum and maximum mean

baseline values for body weight, glucose, free fatty acids, insulin and HOMA for each of the 11 treatment groups were as follows: 592-651 g; 4.98-10.35 mmol/l; 0.67-1.68 mmol/l; 13.3-45.3 μIU/ml; 3.3-18.2. For each of these variables, except body weight, ANOVA demonstrated a statistically significant difference across the 9 groups of animals.Therefore, to correct for the differences observed at baseline, the data collected at weeks 1, 2 and 3 was expressed as a % of the baseline values

Statistical analysis, within treatment groups, of data collected at baseline, 1, 2 and 3 weeks was carried out using repeated measures ANOVA. Analysis of data across treatment groups at each time point was performed using a 1-way ANOVA. The post hoc test used for comparing paired means after both ANOVAs, was Tukey's test. This test was chosen as it reduces the risk of type 1 errors occurring.

The AUC was calculated for all data collected over the 3 week treatment period and values were compared between normal diet fed and high calorie diet fed animals using a 2-way ANOVA. This analysis was used to determine if any interaction occurs between the treatments and the diet for each measured variable i.e. food intake, body weight, glucose, FFA, insulin and HOMA levels. Each treatment group was compared with the control group in each of the 2 different diet groups.

Although much of the data displayed a non-Gaussian distribution analysis of the mean values with parametric or non-parametric statistical tests gave very similar

results. The p-values given in the Results section all come from the use of parametric statistical analyses.

Statistica v9.1 (StatSoft, Tulsa, OK, USA) was used for all statistical analyses.

CHAPTER 4 RESULTS

4.11Toxicity tests

After the 3 week period of dosing with the plant extracts all the rats in the two toxicity tests showed no signs of distress, discomfort and no deaths were recorded.All the animals were adjudged healthy by the animal house veterinarian. Approval was granted for the commencement of the work.

4.12 Extraction of plant extracts

The maceratedpart of each plant was extracted to obtain the crude extract when needed or when the previous extract had been used up. In this study only 2 batches were required to conclude the experiments.

Table 4.1. Extraction percentage for each plant extract.

Plant	Batch	Dried plant weight (g)	Weight of extract (g)	Extraction percentage (%)
IT tuber	a	93.46	41.61	44.52
	b	145.15	66.50	45.81
Mean		**119.31**	**54.06**	**45.17**
STD		36.55	17.60	0.91
Ananas cosmos fruit	a	95.37	33.48	35.11

	b	155.55	65.12	41.87
Mean		**125.46**	**49.30**	**38.49**
STD		42.55	22.37	4.78
Uraria picta leaves	a	97.73	30.94	31.67
	b	149.52	55.12	36.84
Mean		**123.63**	**43.03**	**34.26**
STD		36.62	17.10	3.66
Mixed	a	93.52	30.86	33.00
	b	163.50	65.37	40.00
Mean		**128.51**	**48.12**	**36.50**
STD		49.48	24.40	4.95

Table 4.1. Gives the average solute extracted from each dried macerated plant extract and a mixture of the plant extracts. Extracts were carried out in batches indicated as “a” and “b”. As can be seen from table 4.1,the percentage extracted of each plant were very similar for the two batches. Batch “a” was used for the normal diet fed animals and batch “b” was used for the high calorie diet fed animals.

Table 4.1a Differences between HCD and normal diet fed animals at baseline

Variable	Normal diet fed rats (N=53)	High calorie diet fed rats (N=66)
Body weight (g)	643 ± 55	623 ± 55*
Fasting glucose (mmol/l)	6.30 ± 1.11	7.17 ± 2.29*
Fasting insulin (ulU/ml)	4.98 ± 1.67	33.0 ± 15.0***

Data is expressed as mean ± SD; *p<0.05, ***p<0.0005 versus normal diet fed rats

Table 4.1a shows differences at baseline, for body weight, fasting glucose and fasting insulin concentration between the normal diet fed and the high calorie diet fed animals. Body weight was slightly higher in the normal diet fed animals whilst fasting glucose and insulin concentration were significantly higher in the high calorie diet fed animals.

4.1 Weight of rats during the period of dosing in normal fed and HCD diet fed rats.

Figures 4.1.1 – 4.1.4 show the median percentage weight of the normal diet fed animals (A) and the high calorie diet fed animals (B) for control animals and all treatment groups.

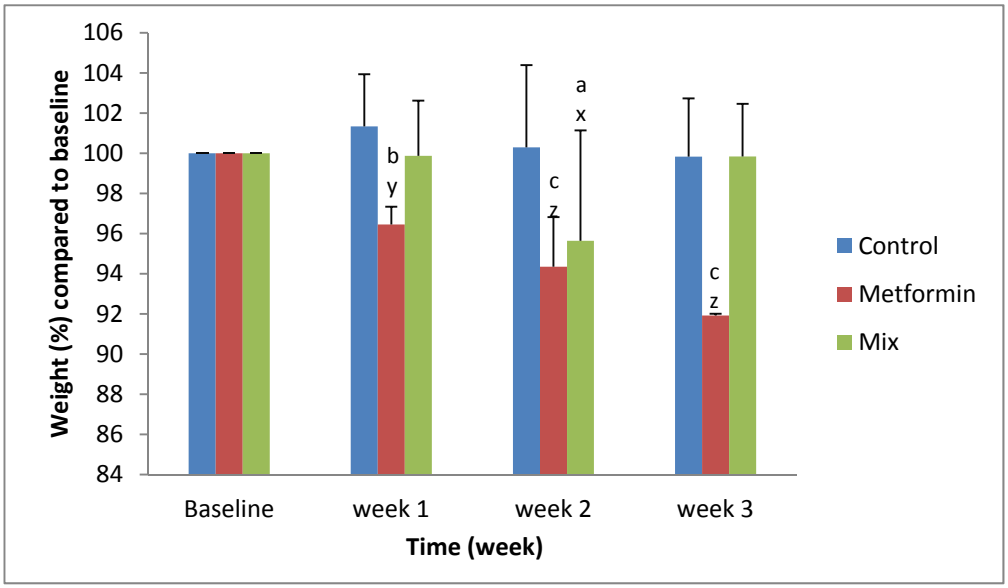

Figure A: Normal diet fed

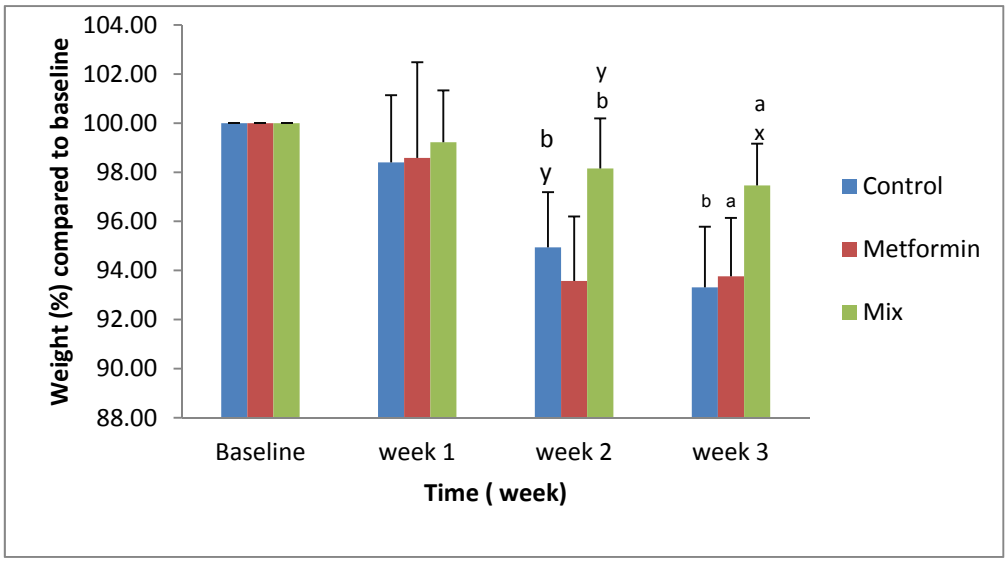

Figure B: High calorie fed

Figure 4.1.1. Bar chart showing the percentage median weight in the normal diet fed animals (A) and the high calorie diet fed animals (B) in control, metformin and mixture groups of rats over 3 weeks of dosing with the of plant extracts.
[a]p<0.05, [b]p<0.005, [c]p<0.0005 vs baseline .[x]p<0.05, [y] p<0.005, [z]p<0.0005 vs controls.

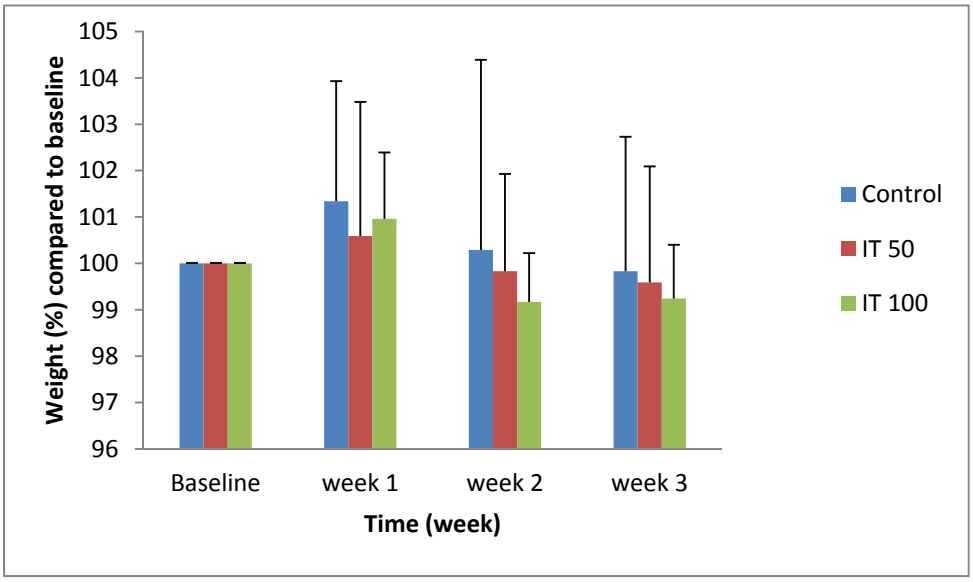

Figure A: Normal diet fed

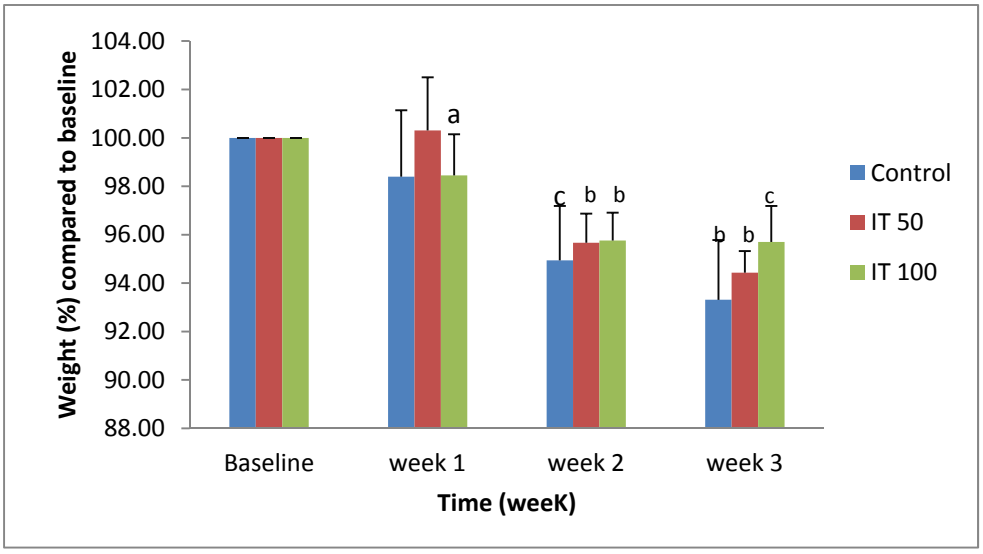

Figure B: High calorie fed

Figure 4.1.2 Bar chart showing the percentage median weight in the normal diet fed animals (A) and the high calorie diet fed animals (B) in control, IT50 and IT100 groups of rats over 3 weeks of dosing.
[a]p<0.05, [b]p<0.005, [c]p<0.0005 vs baseline
[x]p<0.05, [y] p<0.005, [z]p<0.0005 vs controls.

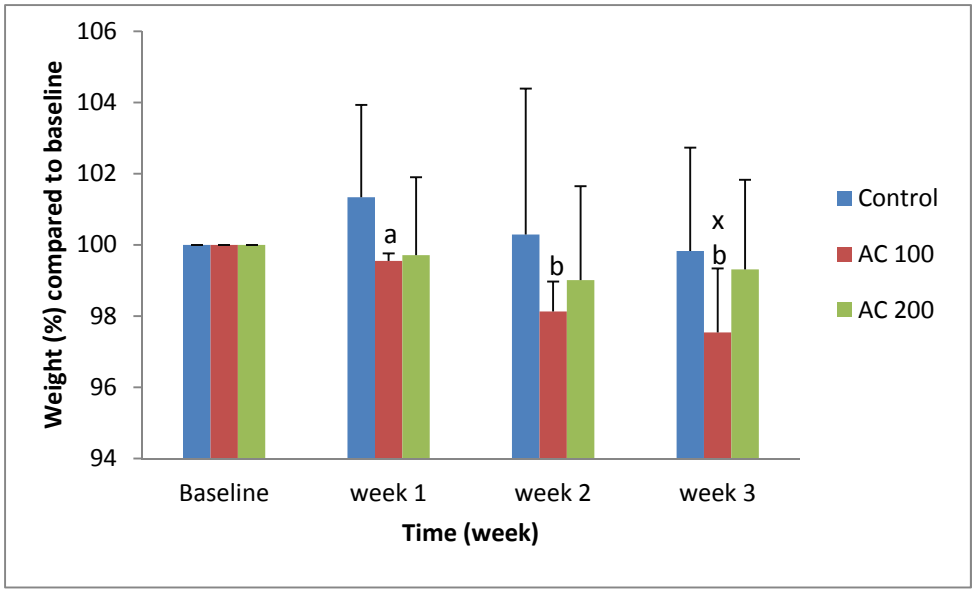

Figure A: Normal diet fed

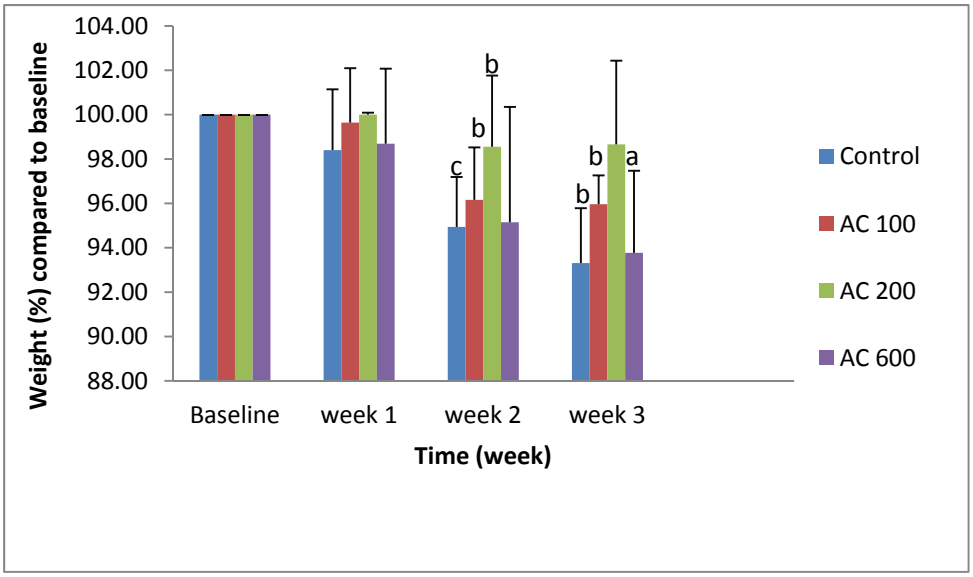

Figure B: High calorie fed.

Figure 4.1.3. Bar chart showing the percentage median weight in the normal diet fed animals (A) and the high calorie diet fed animals (B) in control, AC100, AC200 and AC600 groups of rats over 3 weeks of dosing.
[a]p<0.05, [b]p<0.005, [c]p<0.0005 vs baseline
[x]p<0.05, [y] p<0.005, [z]p<0.0005 vs controls.

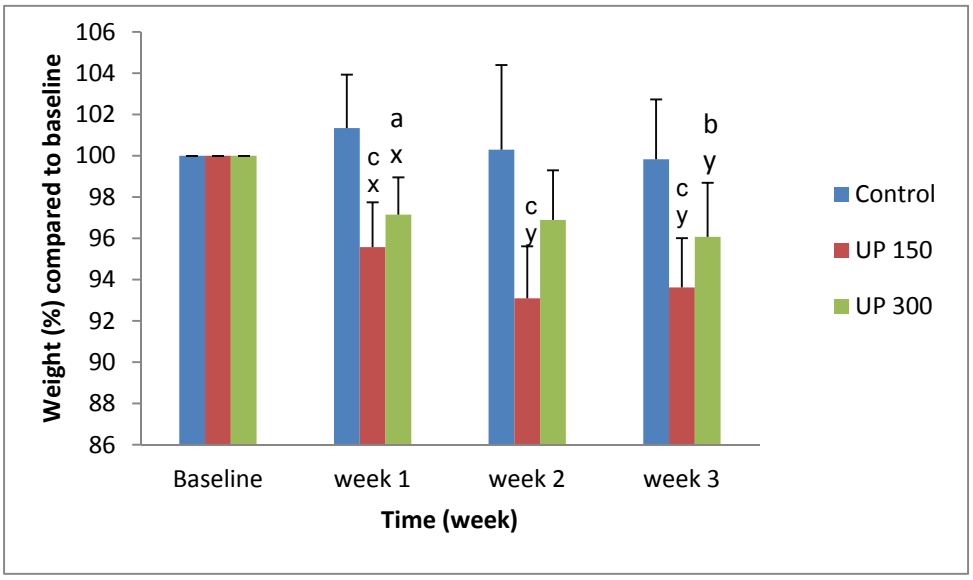

Figure A: Normal diet fed

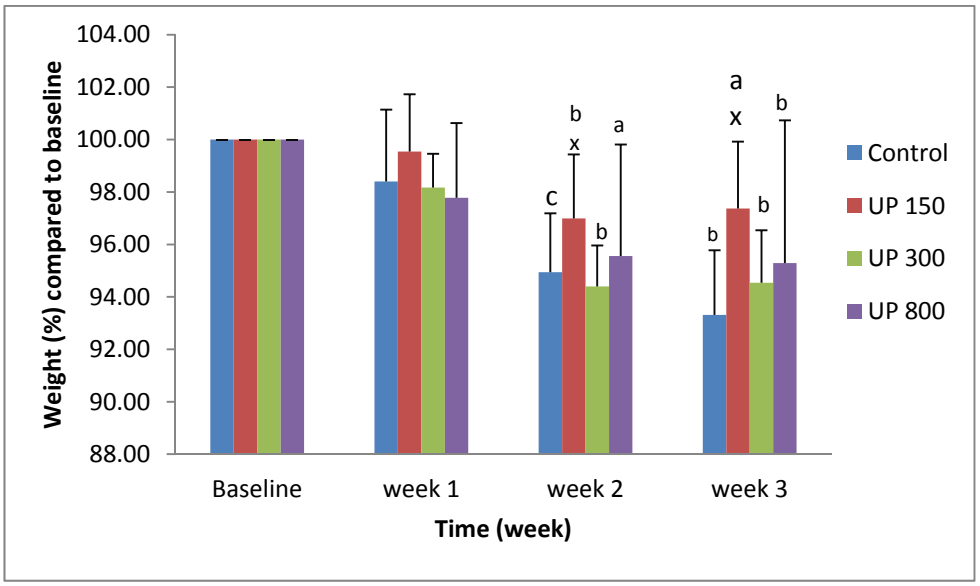

Figure B: High calorie fed

Figure 4.1.4 Bar chart showing the percentage median weight in the normal diet fed animals (A) and the high calorie diet fed animals (B) in control, UP150, UP300 and UP800 groups of rats over 3 weeks of dosing

[a]p<0.05, [b]p<0.005, [c]p<0.0005 vs baseline
[x]p<0.05, [y]p<0.005, [z]p<0.0005 vs controls.

4.1 Description of body weight results.

In figure 4.1.1, the body weight of the normal diet fed rats did not change with time whilst in the high calorie diet animals, body weight fell. Metformin was observed to significantly bring down the weight of the animals in the normal diet fed group of animals. While there was weight reduction with metformin in the high calorie diet, this was not significantly different to that observed in the control animals. The mixed extract brought about significant weight loss in the second week when compared with the control and baseline data in the normal diet fed rats. In the high calorie diet fed animals, the mixed extracts significantly reduced the weight of the animals in the second and third weeks when these values are compared to baseline concentration but at both these time points, weights were higher than in the control animals. These results are confirmed by 2-way ANOVA where a significant interaction was observed between the mixed plant extract and dietary intake ($F=8.7$, $p=0.008$) on the area-under-the-curve data for body weight (%).

In figure 4.1.2, IT50 and *Icacina tricantha* 100 did not have any effects on growth, in both the normal diet fed and high calorie diet fed animals, when compared to that seen in the control animals.

Figure 4.1.3 shows that in the normal diet group, *Ananas cosmos*100 reduced weight over time but the effect was not significantly different from that seen in the control animals, whilst *Ananas cosmos*200 had a minimal effect. The effect of the *Ananas cosmos*plant extract on growth in the high calorie diet fed group was also minimal,

with no significant effect observed when compared against the control group. Figure 4.1.4 shows that in normal diet fed rats, *Uraria picta* 150 and 300 produced a sustained weight loss in comparison to both baseline levels and control animals. In the high calorie diet fed animals, *Uraria picta* treatment had minimal effects on weight when compared to the control group of animals. This differential effect of *Uraria picta* on weight in the normal diet and high calorie diet fed group fed animals is confirmed by 2-way ANOVA, where significant interactions are found between *Uraria picta* use and diet for both 150 ($F=39.8$, $p<0.0001$) and 300mg ($F=6.2$, $p=0.02$) doses for AUC data for body weight.

4.2 Food intake results in normal fed and HCD diet fed rats.

Figures 4.2.1 – 4.2.4show the median percentage food intake in the normal diet fed animals (A) and the high calorie diet fed animals (B).for control animals and all treatment groups

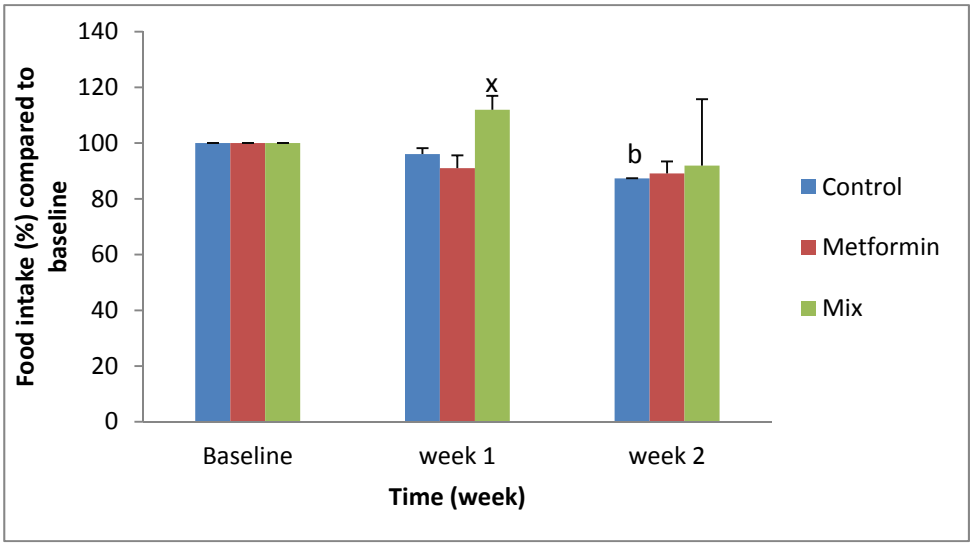

Figure A: Normal diet fed

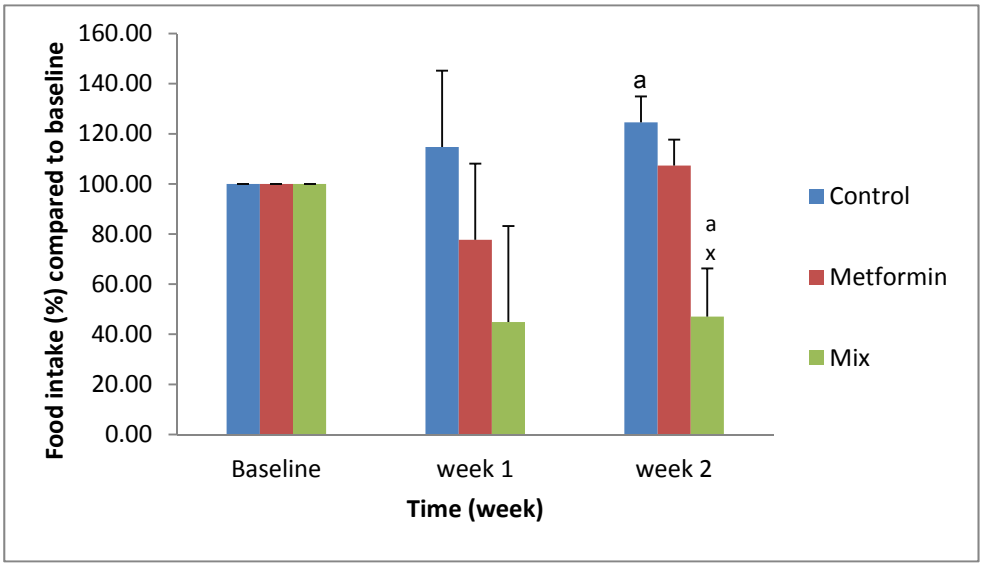

Figure B: High calorie fed

Figure 4.2.1 Bar chart showing the percentage median food intake in the normal diet fed animals (A) and the high calorie diet fed animals (B) in control, metformin and mixed groups of rats over 3 weeks of dosing.

[a]p<0.05, [b]p<0.005, [c]p<0.0005 vs baseline [x]p<0.05, [y] p<0.005, [z]p<0.0005 vs controls.

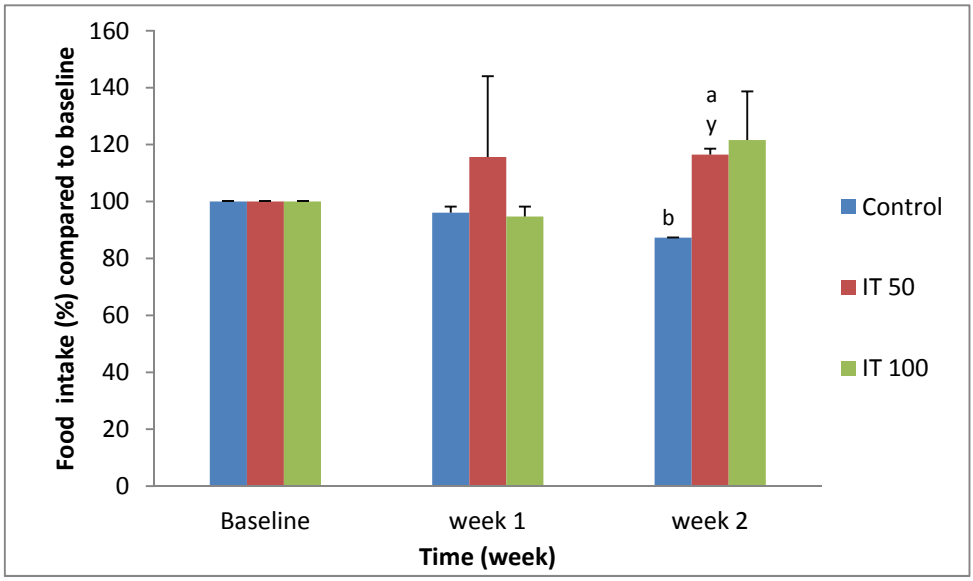

Figure A: Normal diet fed

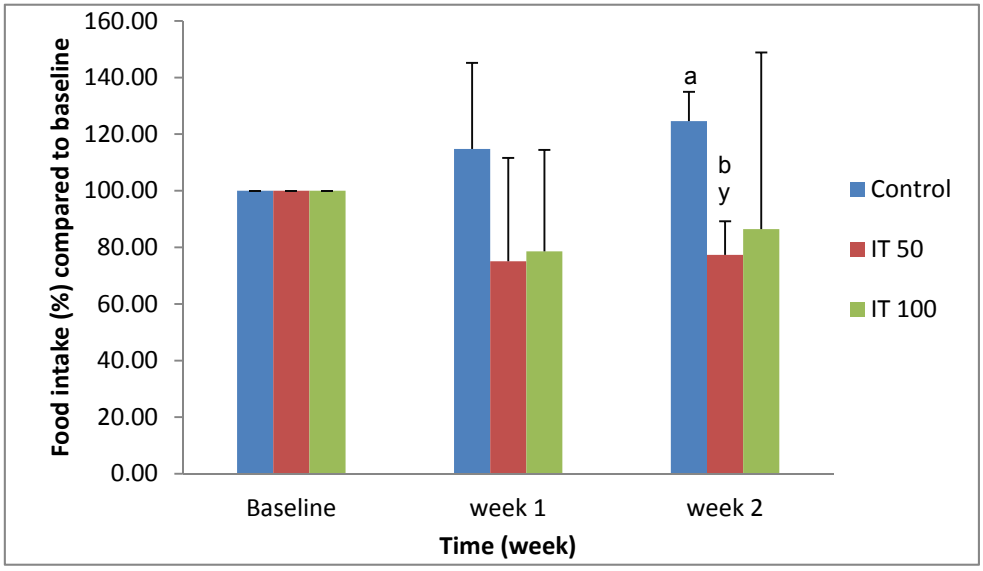

Figure B: High calorie fed

Figure 4.2.2. Bar chart showing the percentage median food intake in the normal diet fed animals (A) and the high calorie diet fed animals (B) in control, IT50 and IT100 groups of rats over 3 weeks of dosing.
[a]p<0.05, [b]p<0.005, [c]p<0.0005 vs baseline
[x]p<0.05, [y]p<0.005, [z]p<0.0005 vs controls.

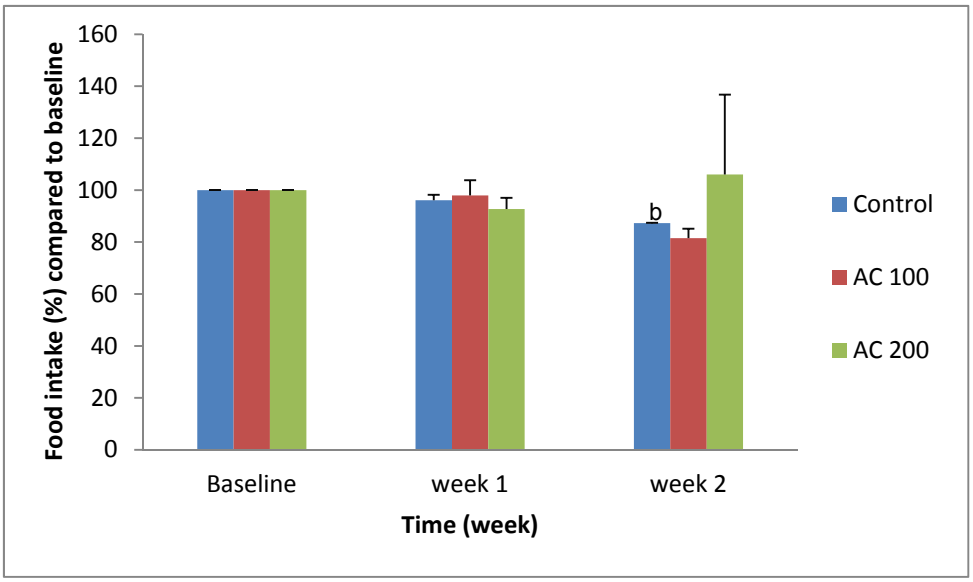

Figure A: Normal diet fed

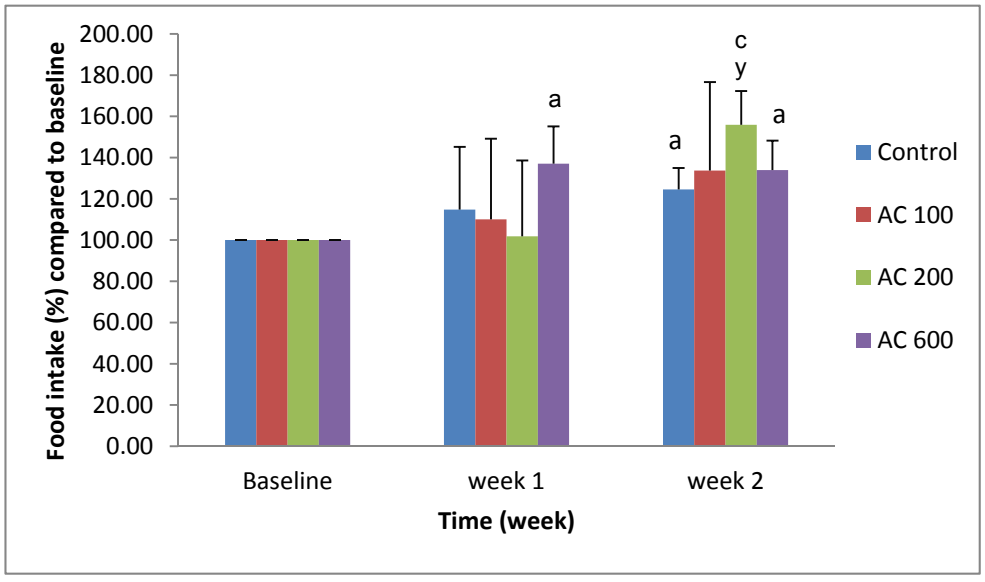

Figure B: High calorie fed

Figure 4.2.3 Bar chart showing the percentage median food intake in the normal diet fed animals (A) and the high calorie diet fed animals (B) in control, *AC*100, AC200 and AC600 groups of rats over 3 weeks of dosing.

[a]p<0.05, [b]p<0.005, [c]p<0.0005 vs baseline
[x]p<0.05, [y] p<0.005, [z]p<0.0005 vs controls.

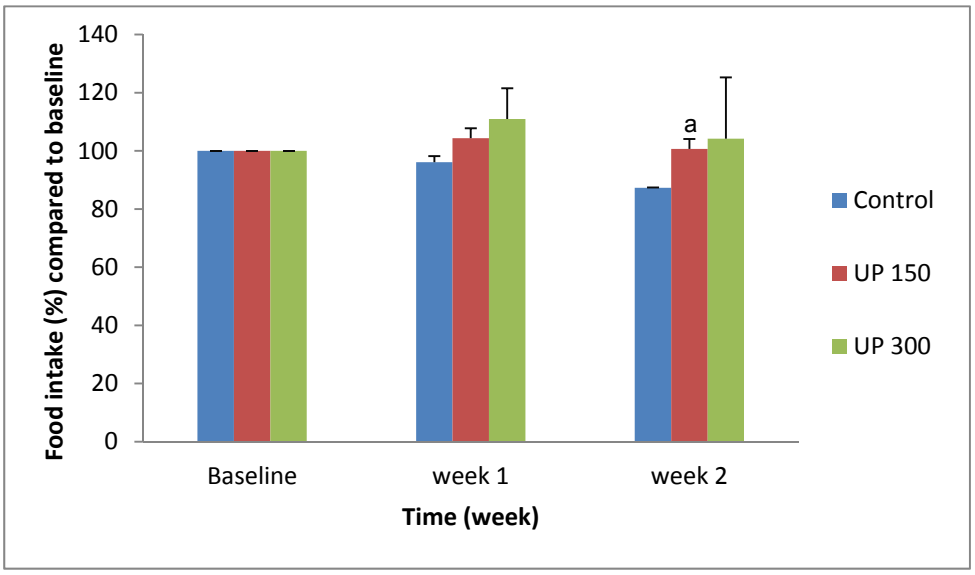

Figure A: normal diet fed

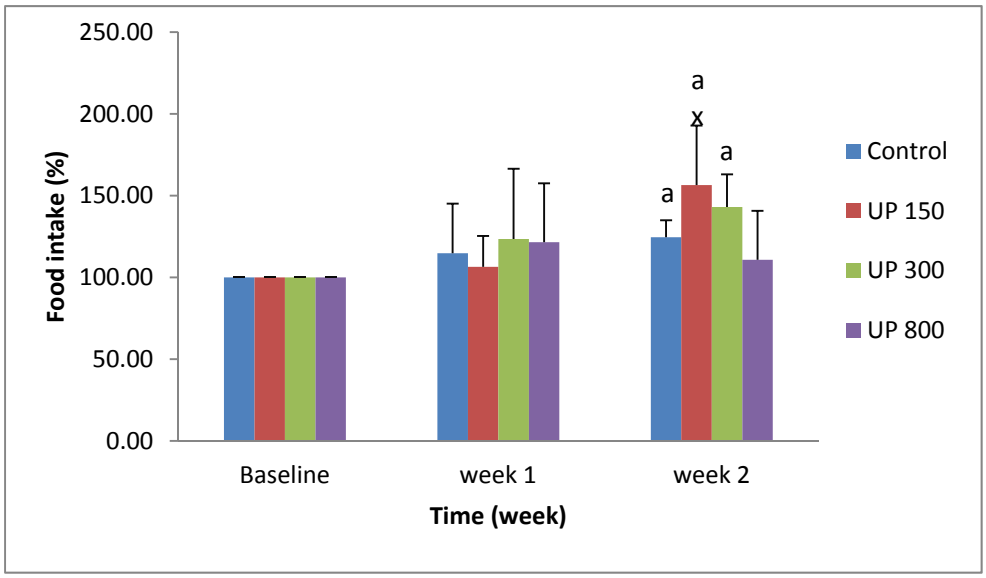

Figure B: High calorie fed

Figure 4.2.4 Bar chart showing the percentage median food intake in the normal diet fed animals (A) and the high calorie diet fed animals (B) in control, UP150, UP300, and UP800 groups of rats over 3 weeks of dosing.

[a]p<0.05, [b]p<0.005, [c]p<0.0005 vs Baseline
[x]p<0.05,[y]p<0.005, [z] p<0.0005 vs controls.

4.2 Description of feeding results.

In figure 4.2.1, food intake fell over time in the control, normal diet fed animals and in the control group of the high calorie diet fed animals. Metformin did not have any significant effect on the quantity of food consumed by the animals receiving the normal diet or the high calorie diet. In the animals fed the mixed plant extracts, food intake in both the high calorie diet and normal diet fed groups was higher than in the controls at week 2 and week 1, respectively.

In figure 4.2.2, animals receiving the normal diet had their food intake increased by IT50 compared to baseline and control concentration by week 2, whilst in the high calorie diet animals *Icacina tricantha* 50 reduced food intake by week 2. These data are confirmed by 2-way ANOVA where a significant interaction between *Icacina tricantha* 50 use and dietary treatment on AUC levels of food intake was observed ($F=14.9$, $p=0.008$). The *Icacina tricantha* 100 had no significant effects.

In Figure 4.2.3, the *Ananas cosmos* plant extract had no effect on food intake in the normal diet fed animals whilst in the high calorie diet animals, food intake was increased by *Ananas cosmos*200 above control concentration by week 2.

In figure 4.2.4, *Uraria picta* 150 increased food intake relative to the control animals in both the normal diet and the HCD fed animals, by week 2.

4.3 Glucose concentrations in normal fed and HCD diet fed rats.

Figures 4.3.1 – 4.3.4 show the median percentage of glucose concentration in the normal diet fed animals (A) and the high calorie diet fed animals (B) for control animals and all treatment groups

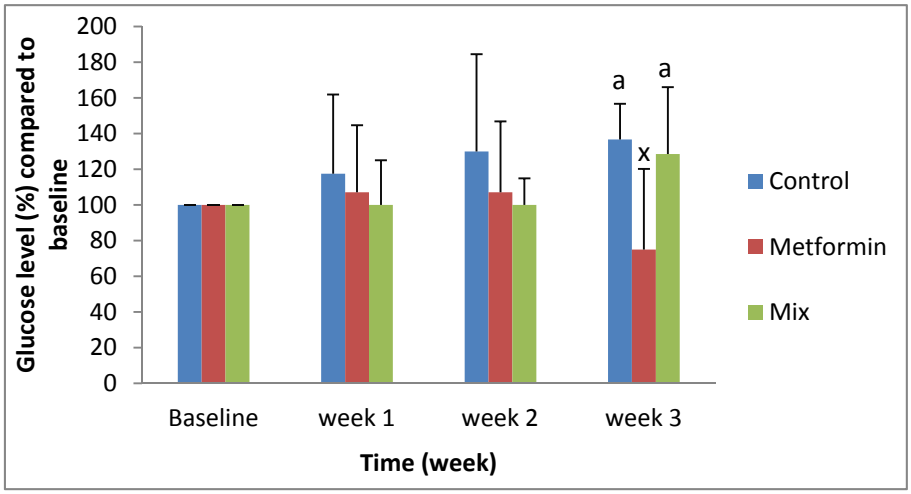

Figure A: Normal diet fed

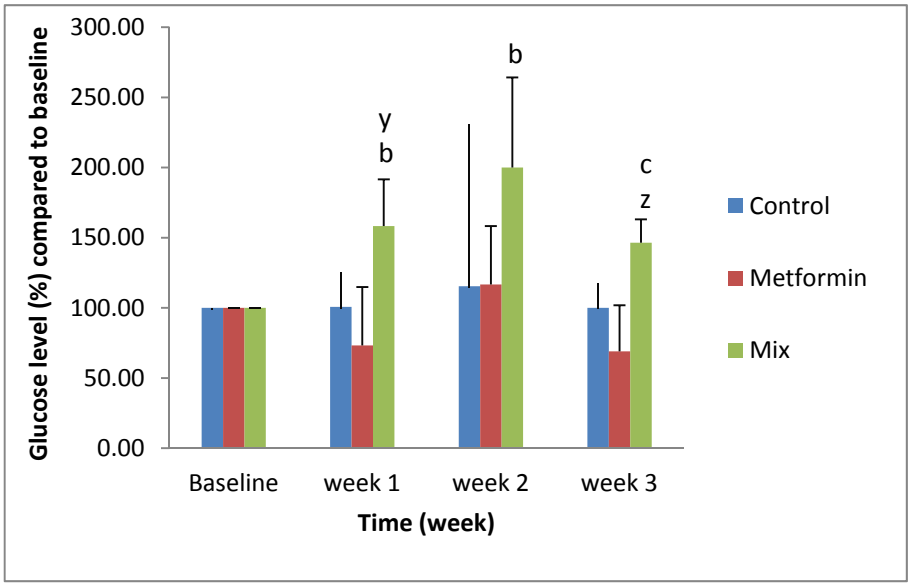

Figure B: High calorie fed

Figure 4.3.1 Bar chart showing the percentage median glucose concentration in the normal diet fed animals (A) and the high calorie diet fed animals (B) in control, metformin and mixed groups over 3 weeks of dosing.
[a]p<0.05, [b]p<0.005, [c]p<0.0005 vs Baseline
[x]p<0.05,[y]p<0.005, [z] p<0.0005 vs controls.

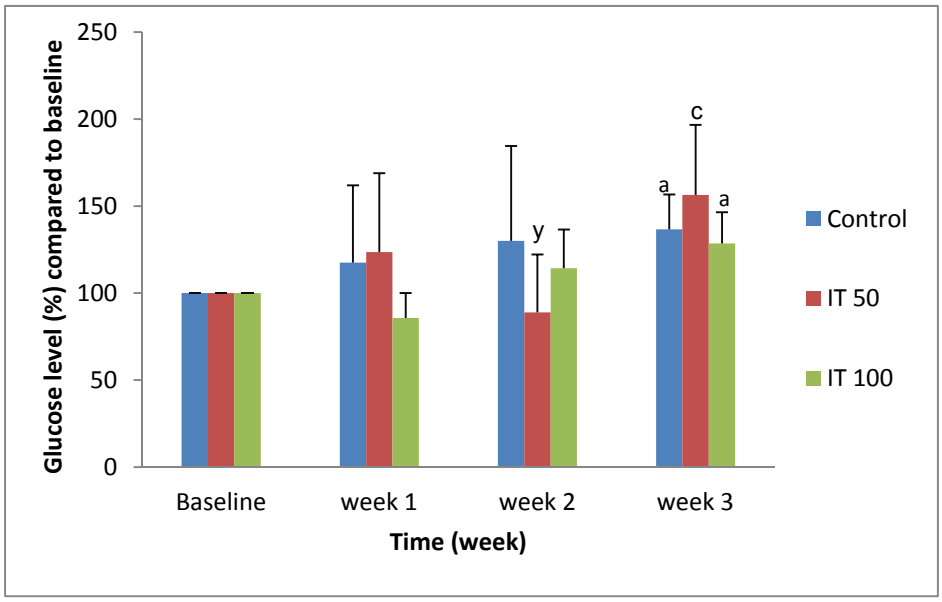

Figure A: Normal diet fed

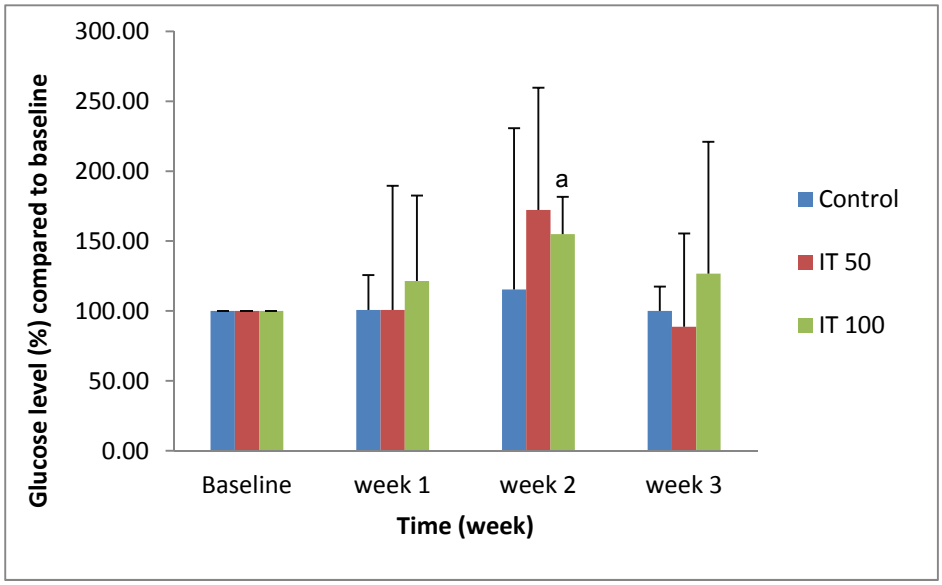

Figure B: High calorie fed

Figure 4.3.2 Bar chart showing the percentage median glucose concentration in the normal diet fed animals (A) and the high calorie diet fed animals (B) in control, IT50 and IT100 groups over 3 weeks of dosing.

[a]p<0.05, [b]p<0.005, [c]p<0.0005 vs Baseline
[x]p<0.05,[y]p<0.005, [z] p<0.0005 vs controls.

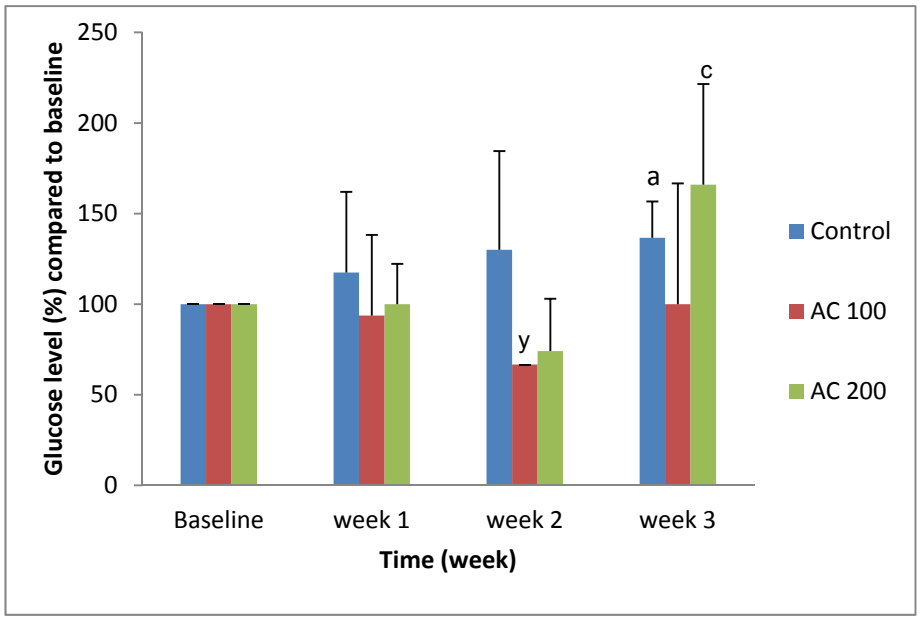

Figure A: Normal diet fed

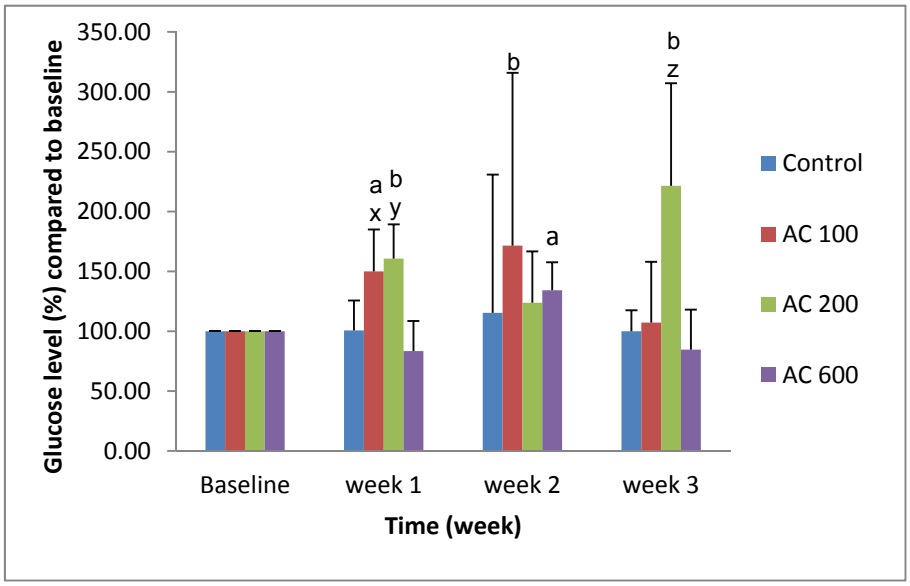

Figure B: High calorie fed

Figure 4.3.3 Bar chart showing the percentage median glucose concentration in the normal diet fed animals (A) and the high calorie diet fed animals (B) in control, AC100, AC200, and AC600 groups over 3 weeks of dosing.

[a]p<0.05, [b]p<0.005, [c]p<0.0005 vs Baseline
[x]p<0.05,[y]p<0.005, [z] p<0.0005 vs controls.

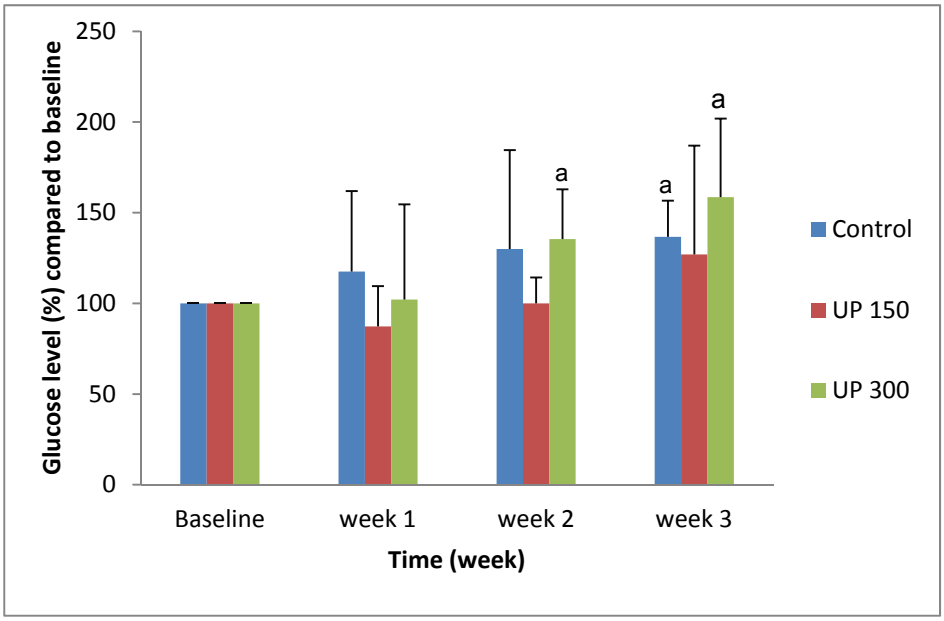

Figure A: Normal diet fed

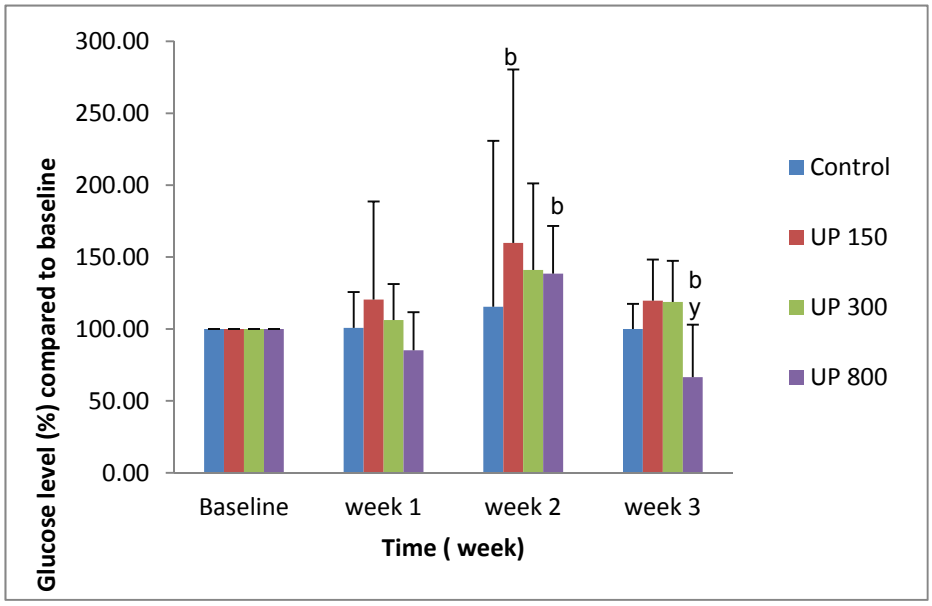

Figure B:High calorie fed

Figure 4.3.4 Bar chart showing the percentage median glucose concentration in the normal diet fed animals (A) and the high calorie diet fed animals (B) in control, UP150, UP300 and UP800 groups over 3 weeks of dosing.

[a]p<0.05, [b]p<0.005, [c]p<0.0005 vs Baseline
[x]p<0.05,[y]p<0.005, [z] p<0.0005 vs controls.

4.3 Description of glucose results.

In figure 4.3.1, glucose concentration rose above baseline concentration in the normal diet fed control animals whilst in the high calorie diet group glucose concentration were not significantly different from baseline values in the control group. In the normal diet fed animals, metformin significantly reduced the blood glucose concentration relative to the control animals by the last week of treatment, but had no significant effect in the high calorie diet fed animals. The mixed plant extract in the normal diet fed animals had no significant effect on glucose concentration when compared to the control animals, whilst the mixed plant extract significantly increased blood glucose concentration in the high calorie diet animals when compared with both baseline and control concentration. This differential effect of the mixed extract on glucose levels is also shown in a 2-way ANOVA where a significant interaction was observed between use of the mixed extract and dietary treatment on AUC glucose levels ($F=15.0$, $p=0.0009$).

In figure 4.3.2, *Icacina tricantha* 50 was shown to reduce glucose concentration relative to control animals in those rodents receiving the normal diet but this effect was seen only at week 2. No effect of either doses of *Icacina tricantha* was observed in the high calorie diet animals relative to the control group.

In figure 4.3.3, in the normal diet fed animals, *Ananas cosmos* 100 reduced blood glucose concentration significantly when compared to the control animals but this effect was observed only at week 2. A similar trend was observed with *Ananas*

cosmos 200 but the 2-week effect was not significant. In the high calorie diet group, *Ananas cosmos*100 and *Ananas cosmos*200 treated animals had blood glucose concentrations that were significantly higher than baseline and control values at week 1 and 3 for *Ananas cosmos*200 and at week 1 for *Ananas cosmos*100. This differential effect of *Ananas cosmos* on blood glucose in the two different diet groups was confirmed by the presence of a statistically significant interaction between *Ananas cosmos* use and AUC data for dietary intake for both the 100mg ($F=12.4$, $p=0.002$) and 200mg ($F=7.2$ and $p=0.01$) doses of *Ananas cosmos.*

Data from figure 4.3.4 show that in the normal diet fed animals the *Uraria picta* plant extract had no significant effect on blood glucose concentration compared with the control animals, whilst in the high calorie diet group, *Uraria picta*800 significantly lowered the glucose concentration compared to the baseline level and the control animals, at week 3. This differential effect of *Uraria picta* on glucose levels in the 2 different diet groups was again confirmed by 2-way ANOVA, where a significant interaction between *Uraria picta*150 use and diet ($F=5.7$, $p=0.03$) for AUC data for glucose, was observed.

4.4 Insulin concentrations in normal fed and HCD fed rats

Figures 4.4.1 – 4.4.4show the median percentage insulin concentration of the normal diet fed animals (A) and the high calorie diet fed animals (B) for control animals and all treatment groups.

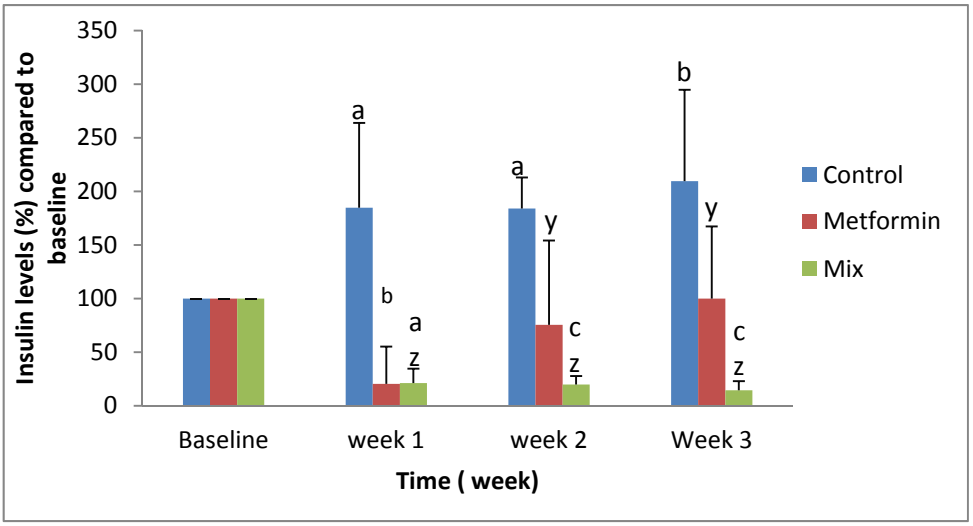

Figure A: Normal diet fed

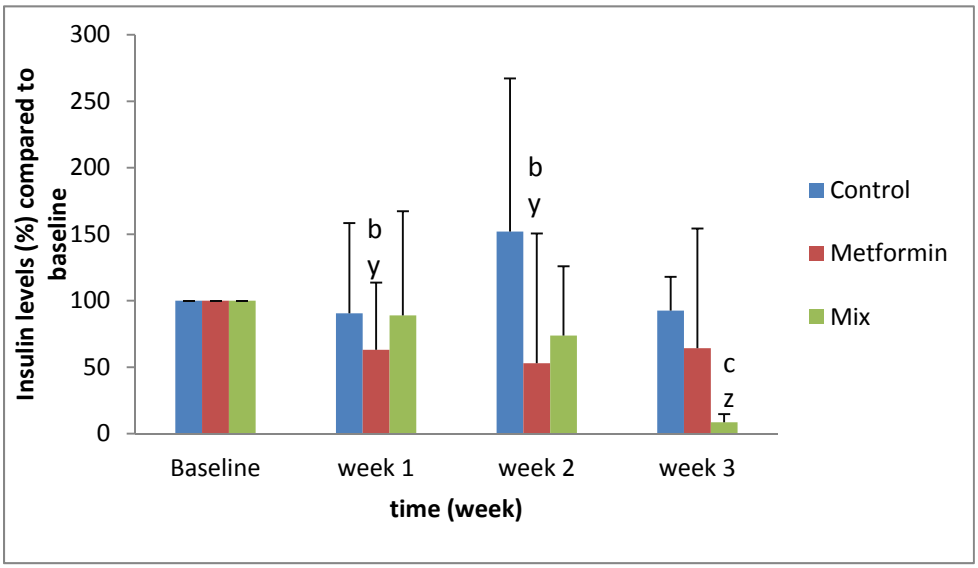

Figure B: High calorie fed

Figure 4.4.1 Bar chart showing the percentage median insulin concentration in the normal diet fed animals (A) and the high calorie diet fed animals (B) in control, metformin and mixed groups over 3 weeks of dosing.

[a]p<0.005, [b]p<0.0005, [c]P<0.0001 vs Baseline [x]p<0.005,y p<0.0005,[z]<0.0001 vs controls.

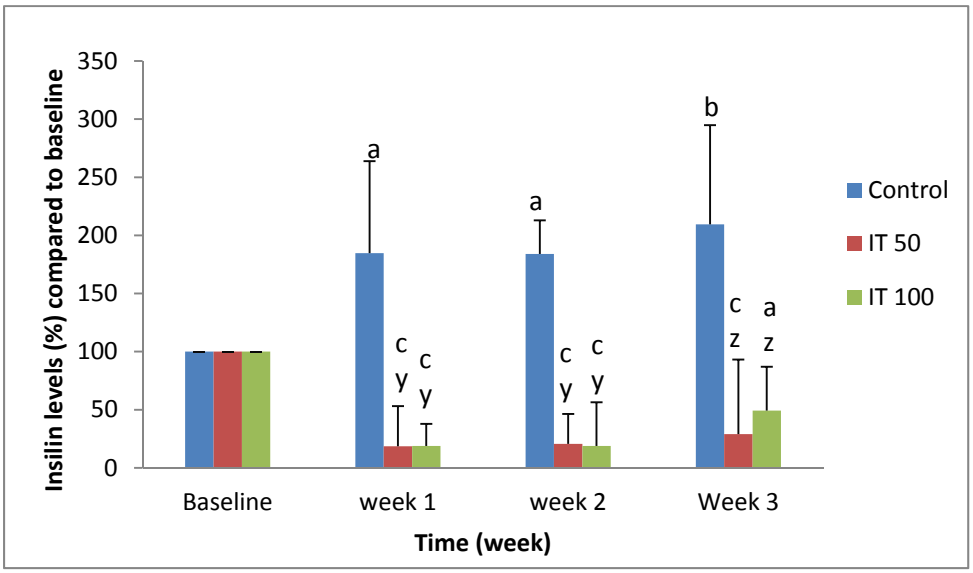

Figure A: Normal diet fed

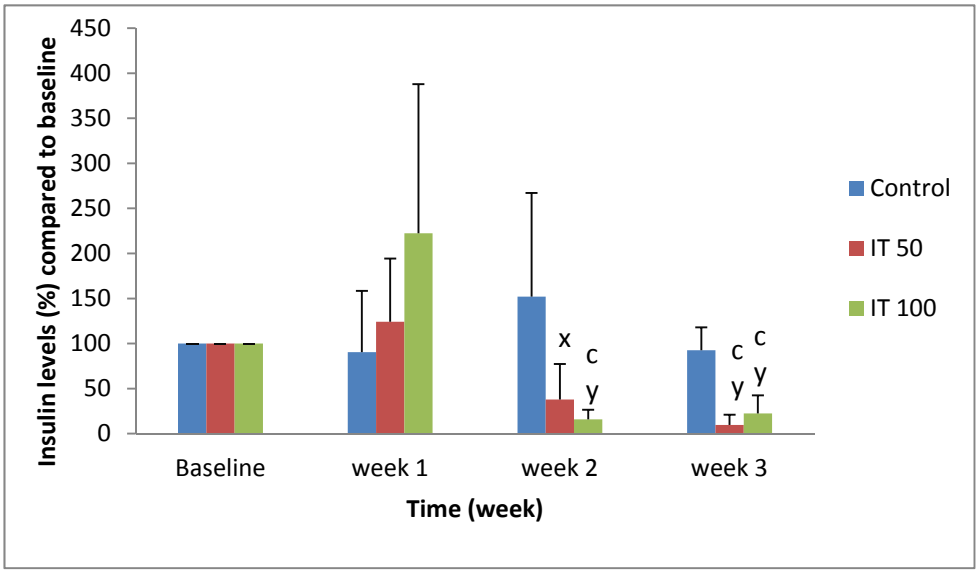

Figure B: High calorie fed

Figure 4.4.2 Bar chart showing the percentage median insulin concentration in the normal diet fed animals (A) and the high calorie diet fed animals (B) in control, IT50 and IT100 groups over 3 weeks of dosing.

[a]p<0.005, [b]p<0.0005, [c]P<0.0001 vs Baseline
[x]p<0.005,y p<0.0005,[z]<0.0001 vs controls

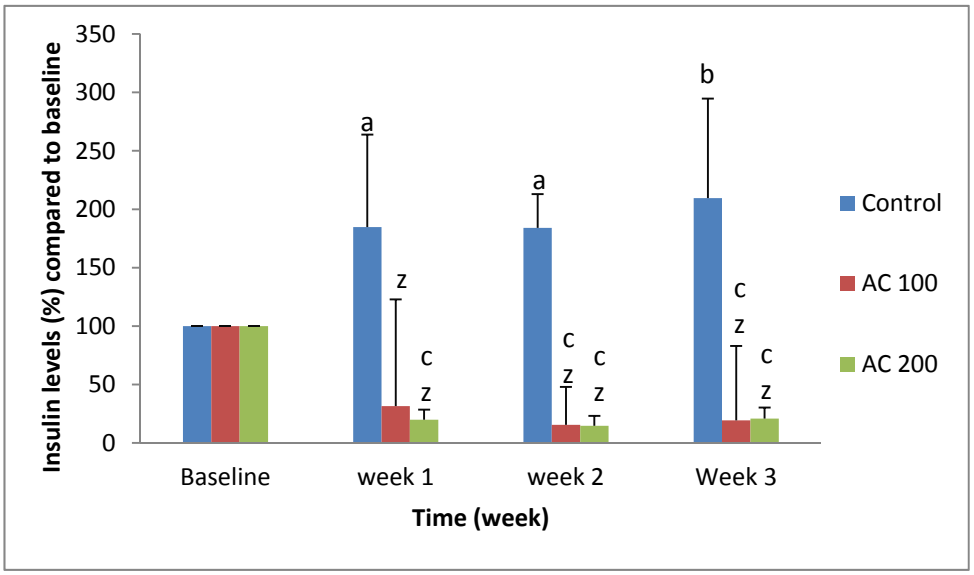

Figure A: Normal diet fed

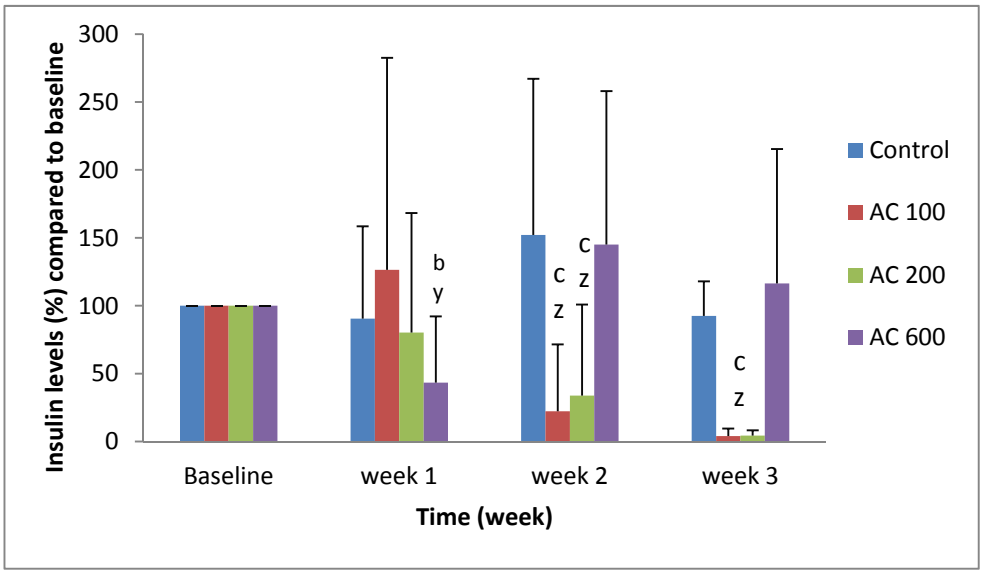

Figure B: High calorie fed

Figure 4.4.3 Bar chart showing the percentage median insulin concentration in the normal diet fed animals (A) and the high calorie diet fed animals (B) in control, AC100, AC200 and AC600 groups over 3 weeks of dosing.
[a]p<0.005, [b]p<0.0005, [c]P<0.0001 vs Baseline
[x]p<0.005,y p<0.0005,[z]<0.0001 vs controls.

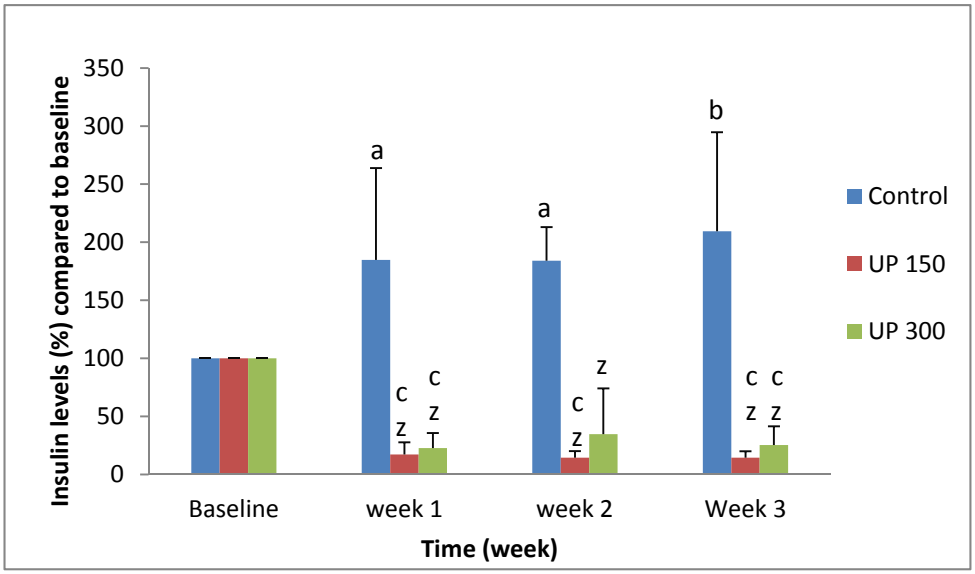

Figure A: Normal diet fed

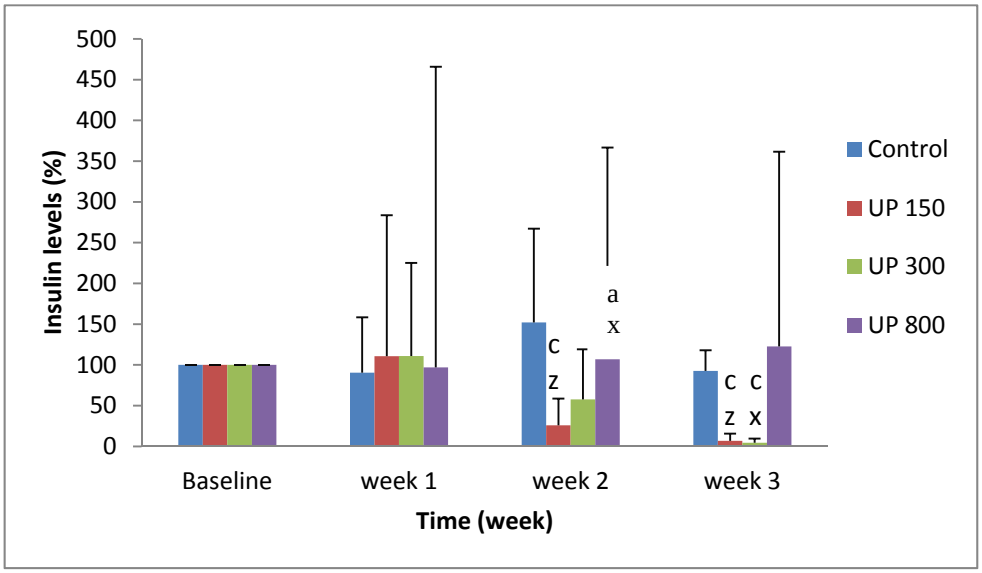

Figure B: High calorie fed

Figure 4.4.4. Bar chart showing the percentage median insulin concentration in the normal diet fed animals (A) and the high calorie diet fed animals (B) in control, UP150, UP300 and UP800 groups over 3 weeks of dosing.

[a]p<0.005, [b]p<0.0005, [c]P<0.0001 vs Baseline
[x]p<0.005,y p<0.0005,[z]<0.0001 vs controls.

4.4 Description of insulin results.

Figure 4.4.1 - 4.4.4 shows the effects of the plant extracts on insulin concentration in both normal fed and high calorie diet fed rats.

Figure 4.4.1 shows that in the normal diet fed animals, insulin concentration increased with time in the control group whilst in the metformin treated group, insulin concentration fell dramatically from baseline concentration and then increased slowly but still remained below control concentration. In the high calorie diet fed animals, insulin concentration did not change significantly over time in the control group whilst in the metformin treated animals, insulin concentration did fall over time and were lower than in the control group but none of the differences were statistically significant.

All the plant extracts in the normal diet fed group produced significant reductions in the concentration of insulin both over time and in comparison to the control animals. In the high calorie diet animals, the mixed plant extracts had no significant effect on insulin concentration (Figure 4.4.1B) whilst *Icacina tricantha* at all doses (Figures 4.4.2B) significantly reduced insulin concentration compared to control animals and baseline concentration at week 2 and 3.The *Ananas cosmos*100 and AC200 extracts significantly reduced insulin concentration relative to control animals and baseline concentration at weeks 2 and 3, whilst AC600 had minimal effects (Figure 4.4.3B).

Figure 4.4.4B shows that in the high calorie diet animals *Uraria picta*150 and *Uraria picta*300 both reduced serum insulin concentration significantly when compared to control animals and to baseline insulin concentration at weeks 2 and 3, but *Uraria picta*800 had no such effect.

All the plant extracts (excluding the mixed extract) seem to reduce insulin levels to a greater extent in the normal diet compared to the high calorie diet fed animals. This is confirmed by 2-way ANOVA where significant interactions between plant extract use and dietary treatment on AUC insulin levels were observed for *Icacina tricantha* 50mg ($F=6.4$, $p=0.02$) and 100mg ($F=5.2$, $p=0.03$), *Ananas cosmos* 100 ($F=4.4$, $p=0.048$) and 200mg ($F=6.3$, $p=0.02$) and Uraria picta 150 ($F=5.8$, $p=0.02$) and 300mg ($F=5.4$, $p=0.03$).

4.5 HOMA values in normal fed and HCD fed rats

Figures 4.5.1 – 4.5.4 show the median percentage of HOMA values of the normal diet fed animals (A) and the high calorie diet fed animals (B) for control animals and all treatment groups

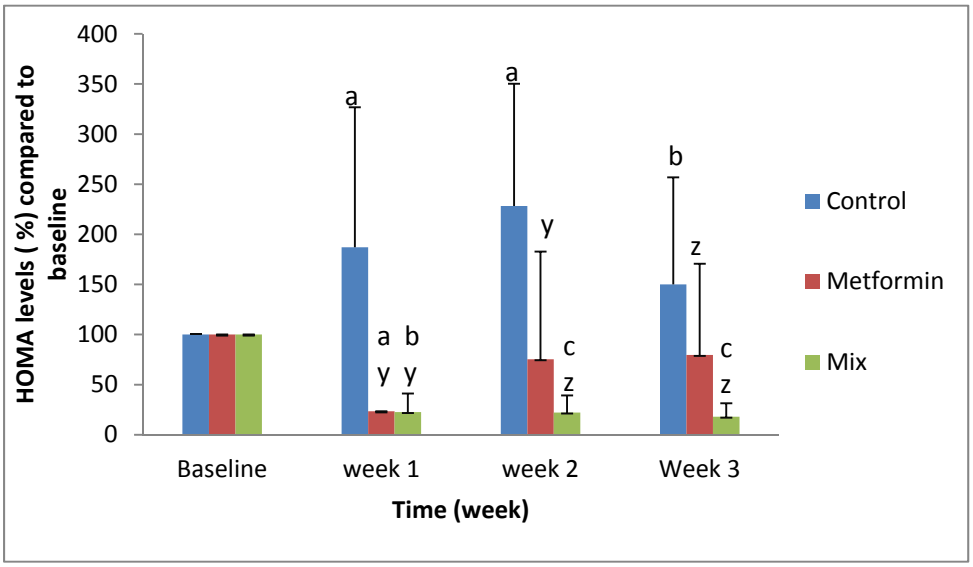

Figure A: Normal diet fed

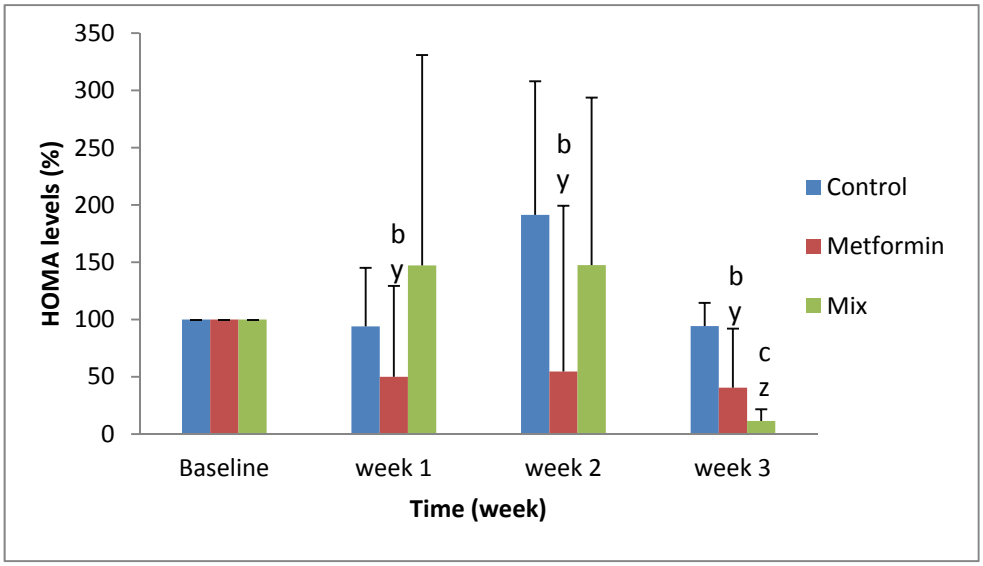

Figure B: High calorie fed

Figure 4.5.1 Bar chart showing the percentage median HOMA values in the normal diet fed animals (A) and the high calorie diet fed animals (B) in control, metformin and mixed plant extracts groups over 3 weeks of dosing.

[a]p<0.005, [b]p<0.0005, [c]P<0.0001 vs Baseline
[x] p<0.005,y p<0.0005,[z]<0.0001 vs controls.

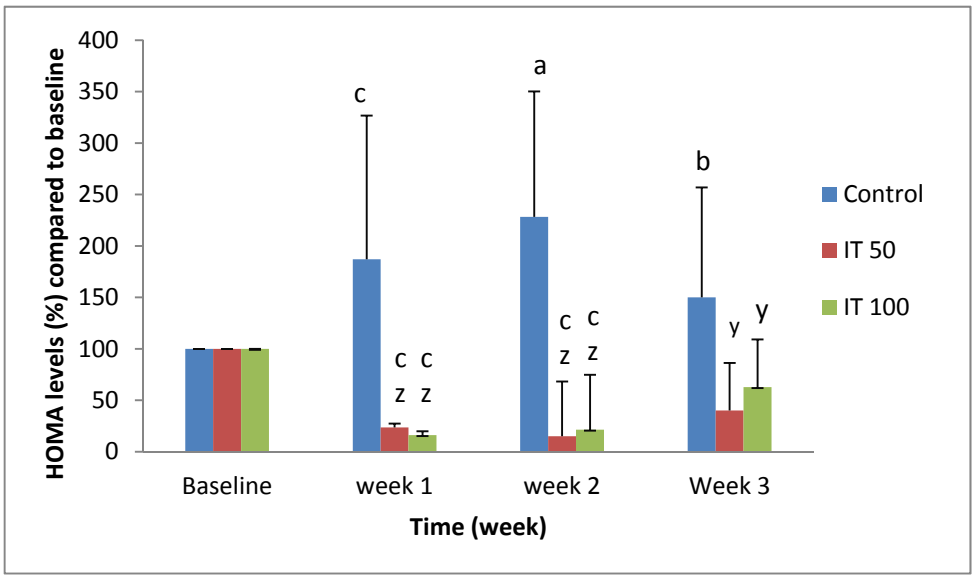

Figure A: normal diet fed

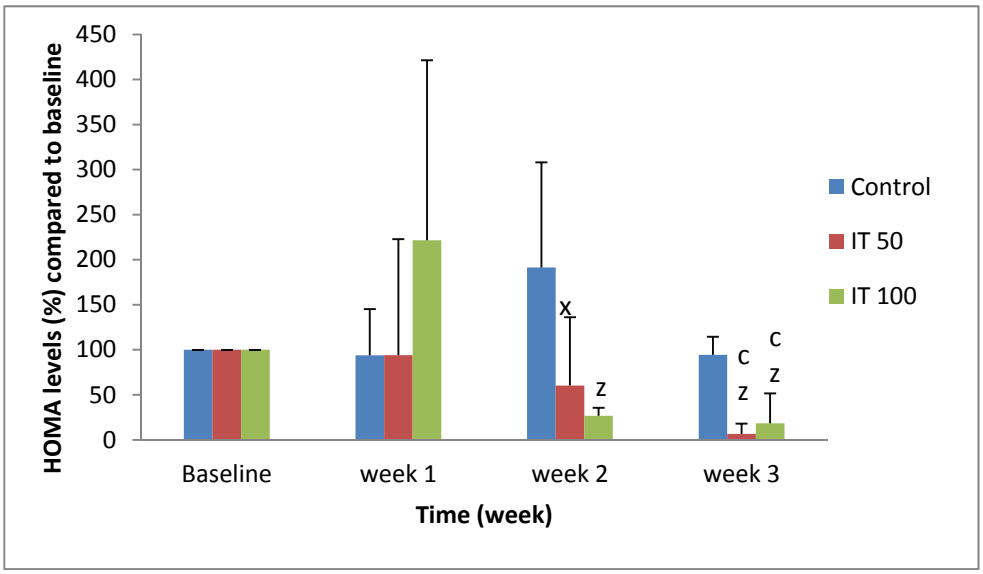

Figure B: High calorie fed

Figure 4.5.2 Bar chart showing the percentage median HOMA values in the normal diet fed animals (A) and the high calorie diet fed animals (B) in control, IT50 and IT100 plant crude extract groups over 3 weeks of dosing.
[a]p<0.005, [b]p<0.0005, [c]P<0.0001 vs Baseline
[x]p<0.005,y p<0.0005,[z]<0.0001 vs controls.

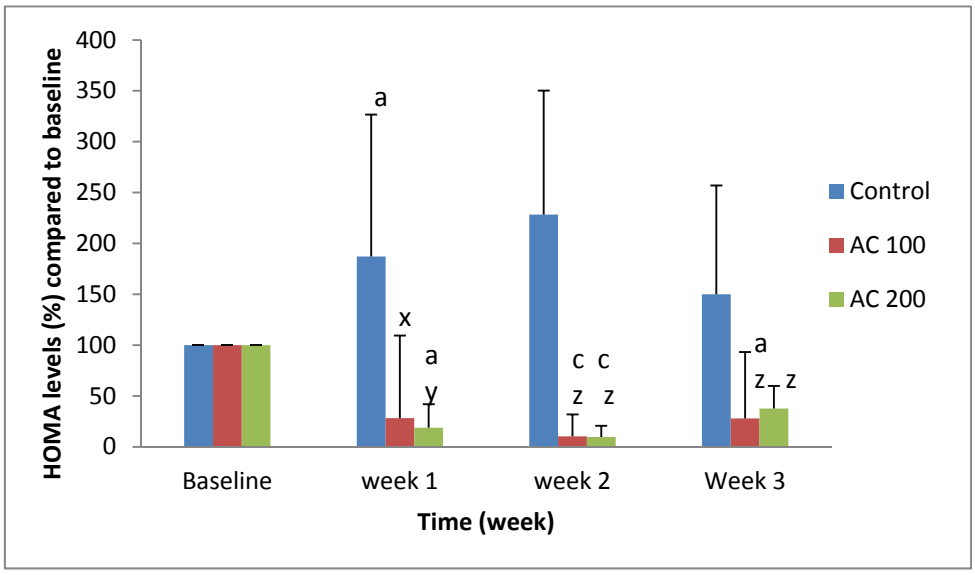

Figure A: Normal diet fed

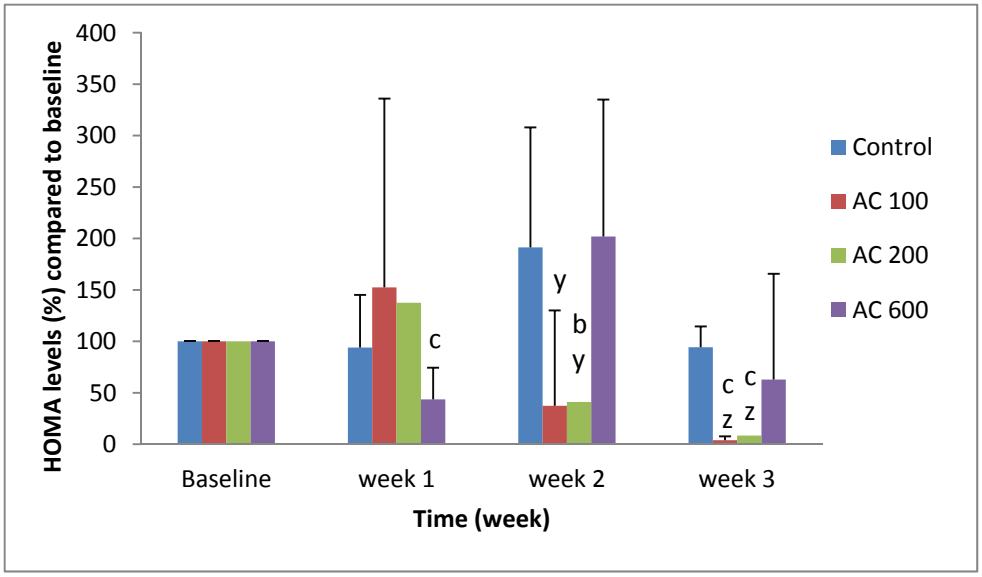

Figure B: High calorie fed

Figure 4.5.3 Bar chart showing the percentage median HOMA values in the normal diet fed animals (A) and the high calorie diet fed animals (B) in control, AC100,AC200 and AC600 doses of crude plant crude extract groups over 3 weeks of dosing.

[a]p<0.005, [b]p<0.0005, [c]P<0.0001 vs Baseline
[x]p<0.005,y p<0.0005,[z]<0.0001 vs controls.

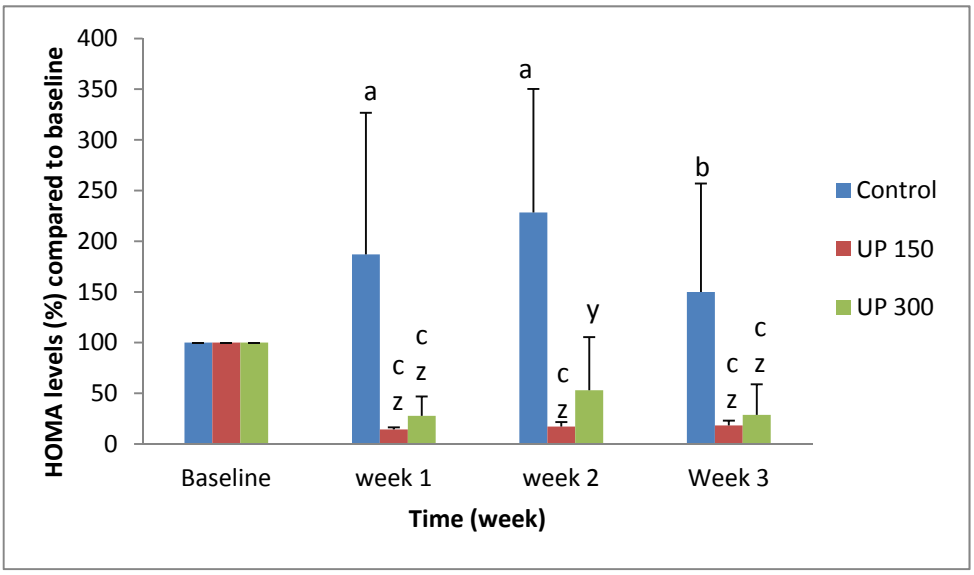

Figure A: Normal diet fed

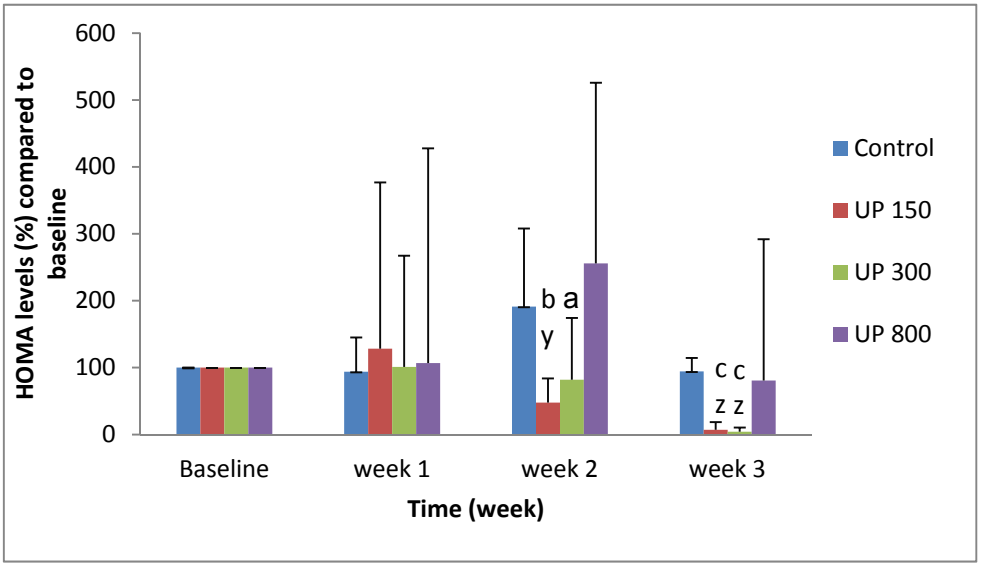

Figure B: High calorie fed

Figure 4.5.4 Bar chart showing the percentage median HOMA values in the normal diet fed animals (A) and the high calorie diet fed animals (B) in control, UP150, UP300 and UP800 doses of crude plant crude extract groups over 3 weeks of dosing.

[a]p<0.005, [b]p<0.0005, [c]P<0.0001 vs Baseline
[x]p<0.005,y p<0.0005,[z]<0.0001 vs controls.

4.5 Description of the effect of plant extract dosing on insulin sensitivity.

Figures 4.5.1 - 4.5.4 describe the effect of the plant extracts on insulin sensitivity. The data for the effects of the plant extracts and metformin on HOMA concentration in both the normal diet and high calorie diet fed animals are exactly the same as those observed for insulin concentrations. This is not surprising, as HOMA and insulin concentration correlate very significantly. The 2-way ANOVA results observed with AUC insulin levels were also replicated for the HOMA AUC data, demonstrating that the plant extracts had greater effects on reducing HOMA in the normal diet than in the high calorie diet fed animals. Thus, significant interactions between extract use and HOMA levels were observed for *Icacina tricantha* 50mg ($F=7.6$, $p=0.01$) and 100mg ($F=4.7$, $p=0.04$), *Ananas cosmos* 100 ($F=7.5$, $p=0.01$) and 200mg ($F=8.4$, $p=0.009$) and Uraria picta 150 ($F=6.3$, $p=0.02$) and 300mg ($F=5.5$, $p=0.03$).

4.6 Free fatty acid concentrations in normal and HCD diet fed rats

Figures 4.6.1 – 4.6.4Shows the median percentage of FFA values of the normal diet fed animals (A) and the high calorie diet fed animals (B) for control animals and all treatment groups.

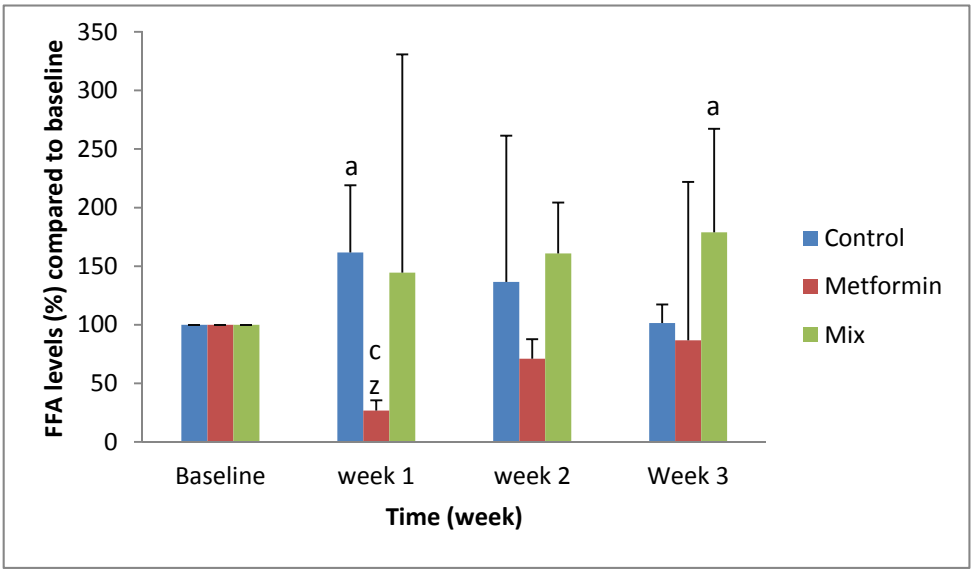

Figure A: Normal diet fed

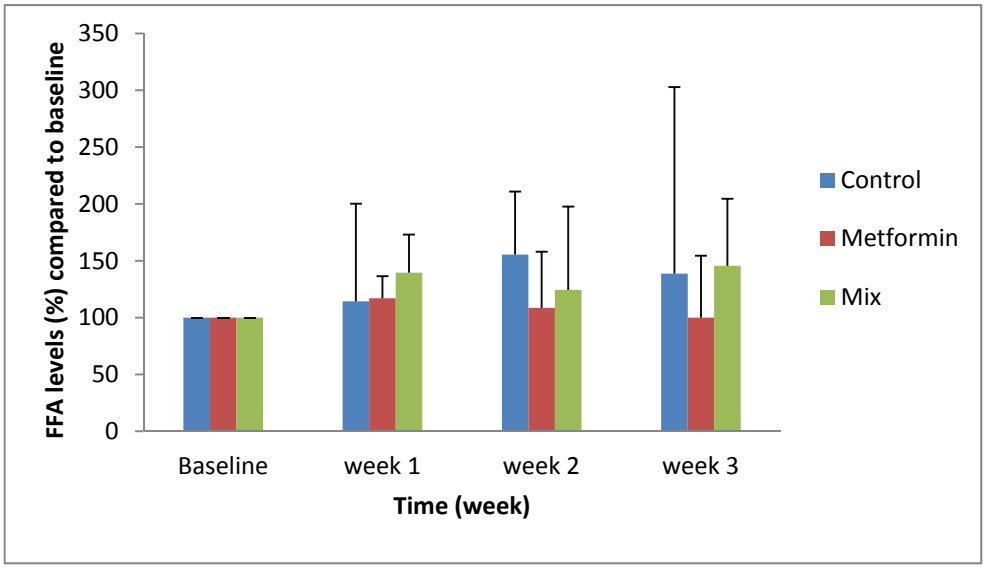

Figure B: High calorie fed

Figure 4.6.1. Bar chart showing the percentage median FFA values in the normal diet fed animals (A) and the high calorie diet fed animals (B) in control metformin and mixed doses of crude extract groups over 3 weeks of dosing.

[a]p<0.05, [b]p<0.005, [c]p<0.0005 vs baseline;
[x]p<0.05, [y]p<0.005 vs control.

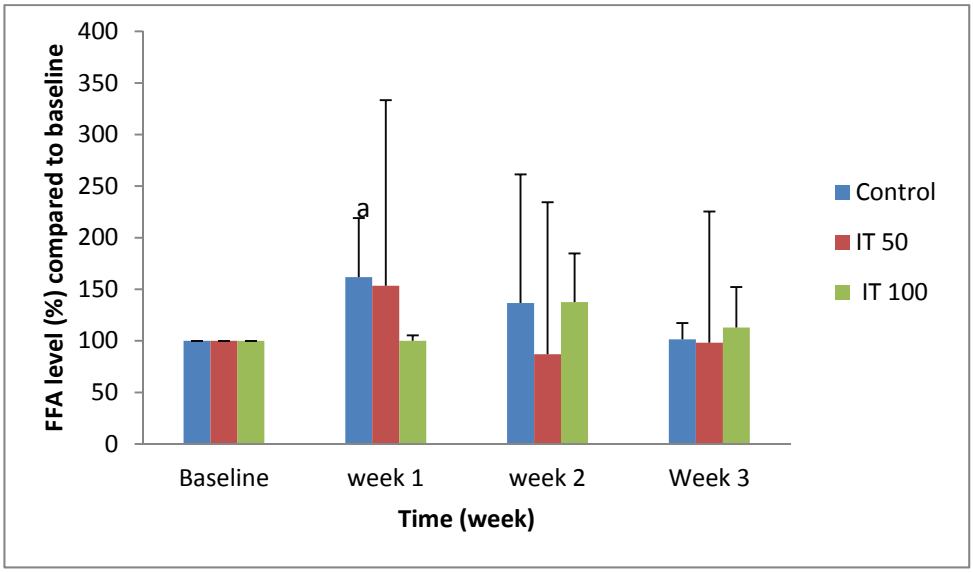

Figure A: Normal diet fed

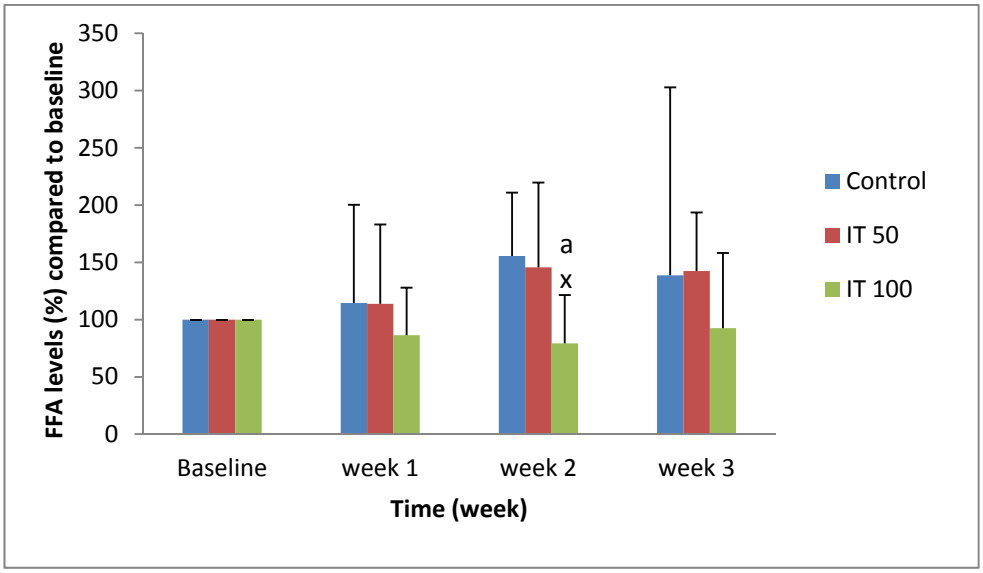

Figure B: High calorie fed

Figure 4.6.2 Bar chart showing the percentage median FFA concentration in the normal diet fed animals (A) and the high calorie diet fed animals (B) in control IT50 and IT100 doses of crude extract groups over 3 weeks of dosing.

[a]p<0.05, vs baseline
[x]p<0.05, vs control.

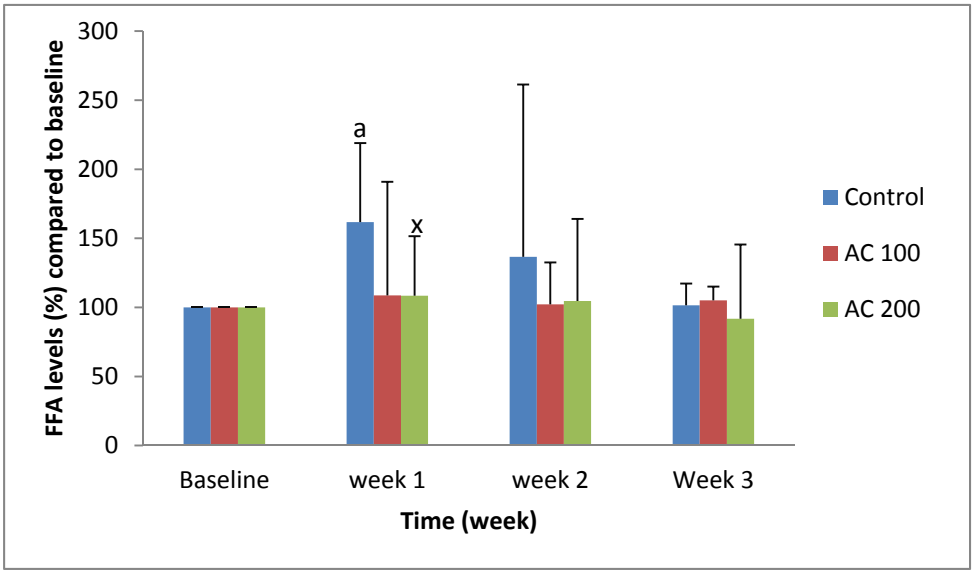

Figure A: Normal diet fed

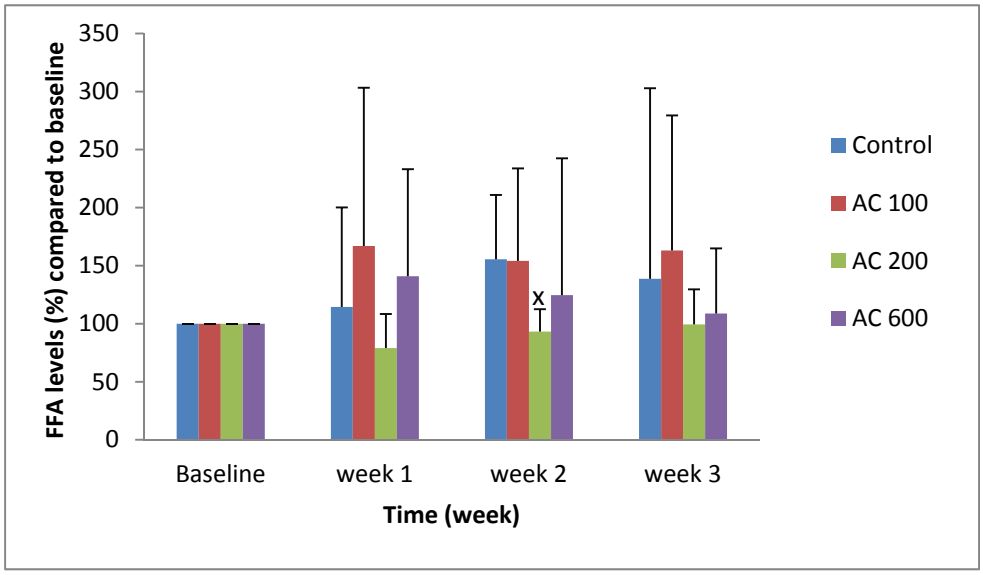

Figure B: High calorie fed

Figure 4.6.3 Bar chart showing the percentage median FFA concentration in the normal diet fed animals (A) and the high calorie diet fed animals (B) in control AC 100, AC200, and AC600 doses of crude extract groups over 3 weeks of dosing.

$^{a}p<0.05$, $^{b}p<0.005$, $^{c}p<0.0005$ vs baseline;
$^{x}p<0.05$, $^{y}p<0.005$ vs control.

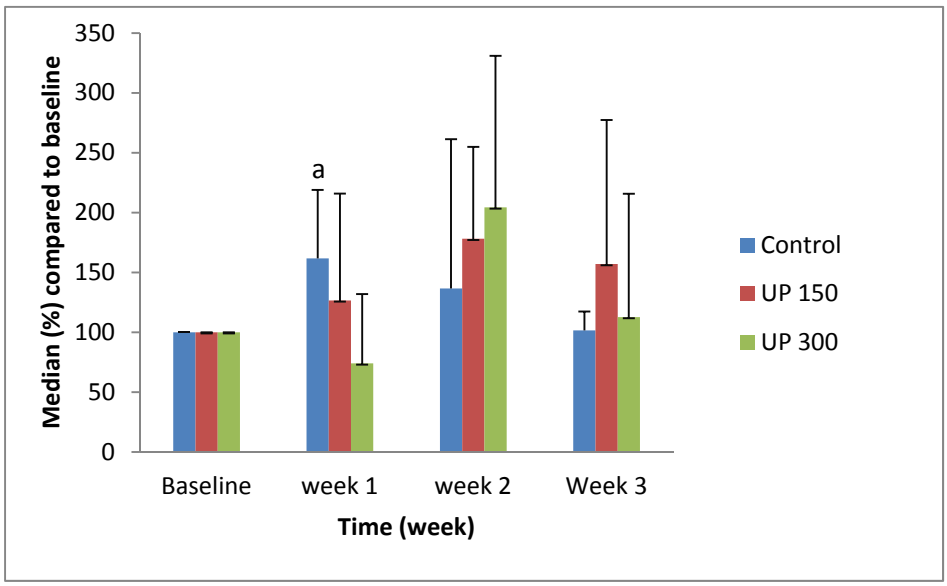

Figure A: Normal diet fed

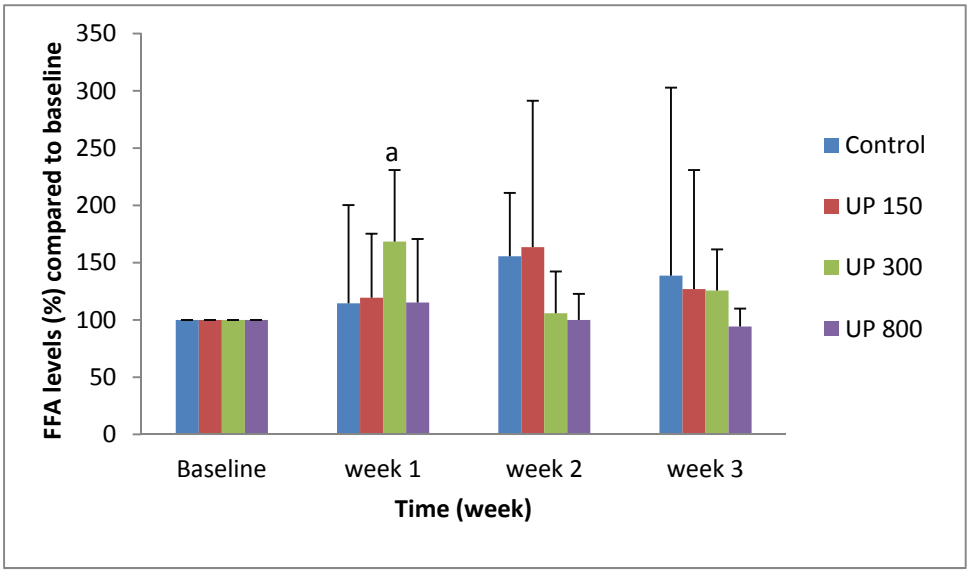

Figure B: High calorie fed

Figure 4.6.4 Bar chart showing the percentage median FFA concentration in the normal diet fed animals (A) and the high calorie diet fed animals (B) in control UP150, UP300, and UP800 doses of crude extract groups over 3 weeks of dosing.

[a]p<0.05, [b]p<0.005, [c]p<0.0005 vs baseline;
[x]p<0.05, [y]p<0.005 vs control.

4.6 Description of free fatty acid (FFA) results.

Figure 4.6.1 shows the serum FFA concentration in the control animals and the effect of metformin and the mixed plant extract on FFA concentration in the normal diet fed and high calorie diet fed animals. In the control animals on the normal diet, FFA concentration rose significantly above baseline values at week 1 but fell back down to baseline concentration by week 3. There was no significant change in FFA concentration over time in the high calorie diet fed control animals. Metformin significantly reduced the concentration of FFA in the normal diet fed ratswhen compared to the control group, but only at week 1. In the high calorie diet fed animals, there was no significant effect of metformin on FFA concentration. The mixed plant extracts had no effect on FFA concentration in either the normal diet fed or high calorie diet fed animals when compared to the relevant control groups.In figure 4.6.2, *Icacina tricantha* 50 and IT100 did not significantly affect the FFA concentration in the normal diet fed animals. The same pattern was observed for the *Icacina tricantha* 50 in the high calorie diet group however, the *Icacina tricantha* 100 extract in the high calorie diet animals significantly reduced the concentration of FFA when compared to the control and baseline values, but only at week 2.

In figure 4.6.3, AC200 in both the normal diet fed and the high calorie diet HCD fed animals significantly reduced FFA concentration compared to control animals at week 1 in the normal diet fed and at week 2 in the high calorie diet fed animals. The other doses of AC had no significant effects.

Figure 4.6.4 shows that the *Uraria picta*extract did not show any significant effect on FFA concentration in either the normal diet fed or the high calorie diet fed animals, when compared to the appropriate control group of animals.

4.7 Oral glucose tolerance test results in normal and HCD diet fed group.

Figures 4.7.1 – 4.7.4 show the OGTT results for the normal diet fed animals (A) and the high calorie diet fed animals (B) for control animals and all treatment groups.

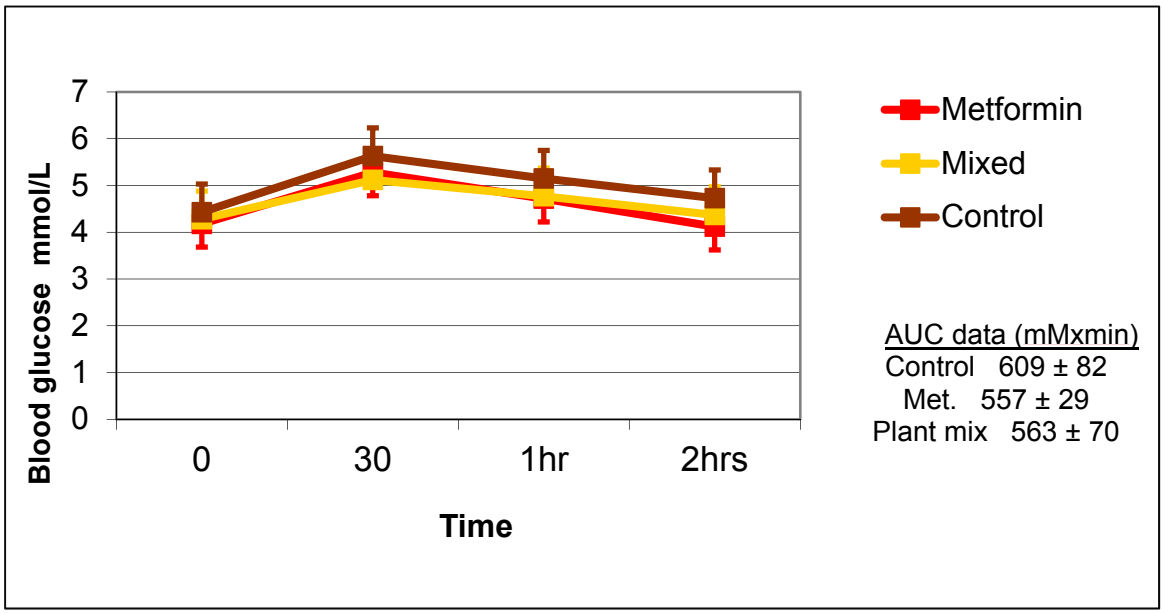

Figure A: Normal diet fed

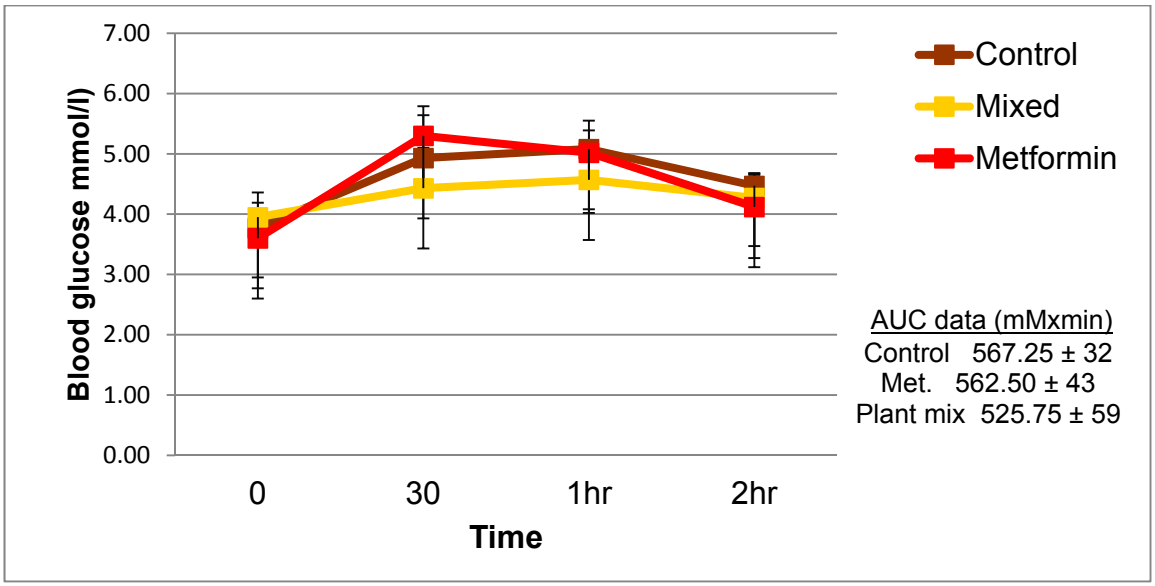

Figure B: High calorie fed

Figure 4.7.1 shows the glucose clearance rate in normal diet fed animal (A) and high calorie diet fed animals(B) in control, metformin and mixed groups

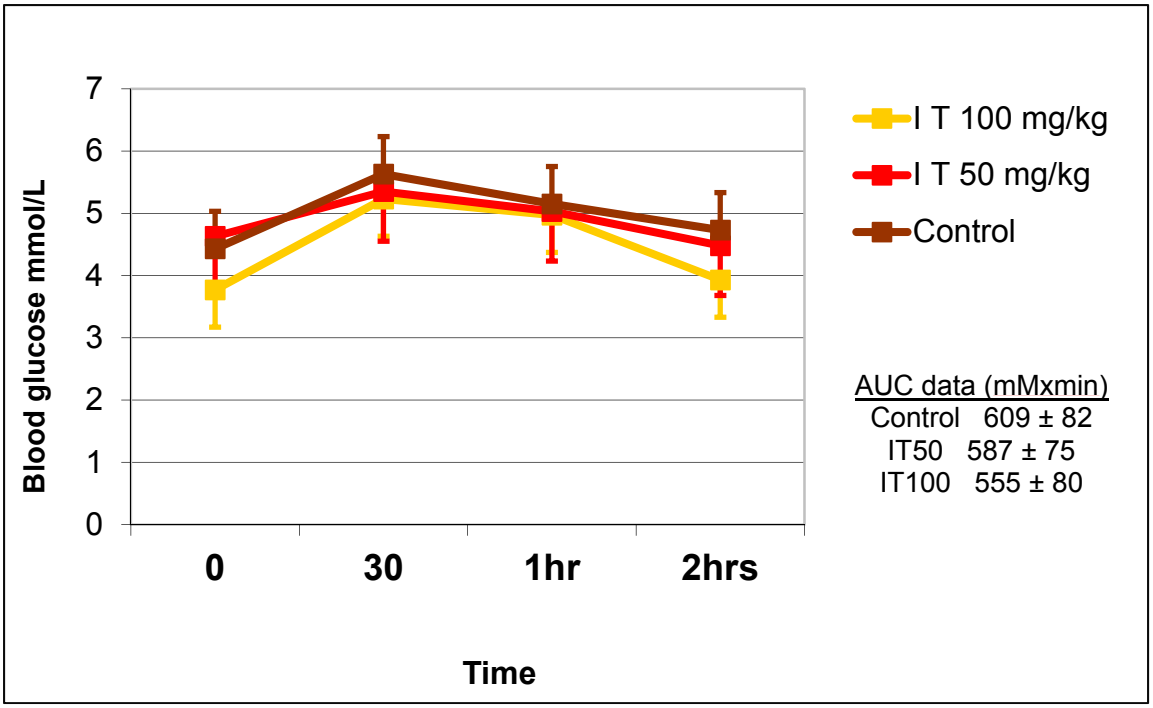

Figure A: Normal diet fed

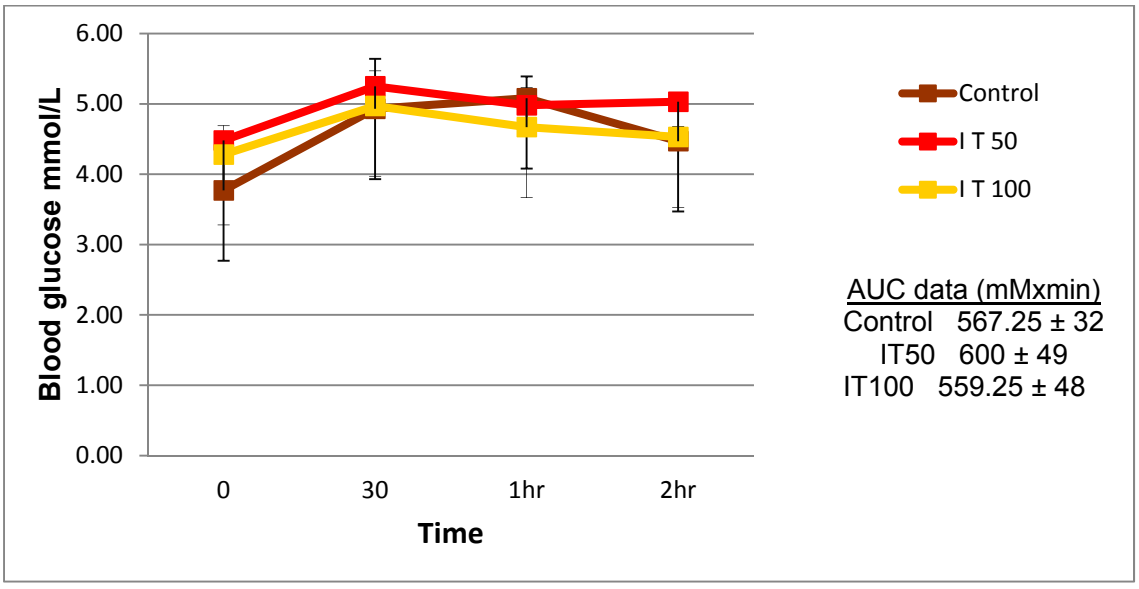

Figure B: High calorie fed

Figure 4.7.2. shows the glucose clearance rate in normal diet fed animal (A) and high calorie diet fed animals(B) in control, IT50 and IT100.

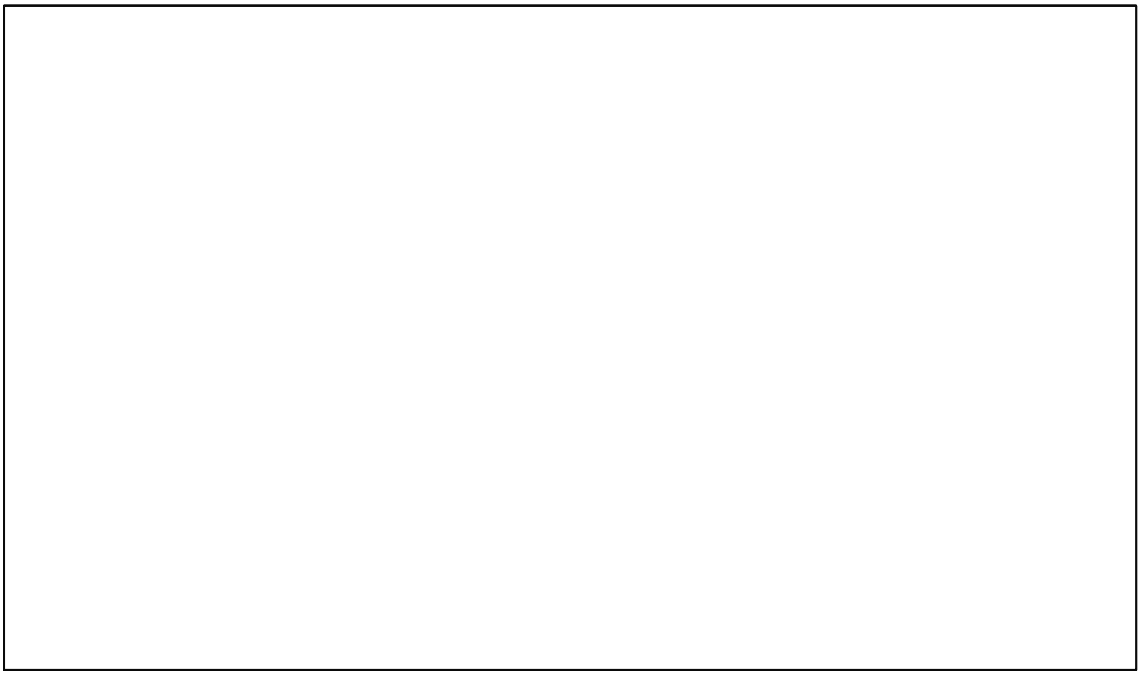

Figure A: Normal diet fed

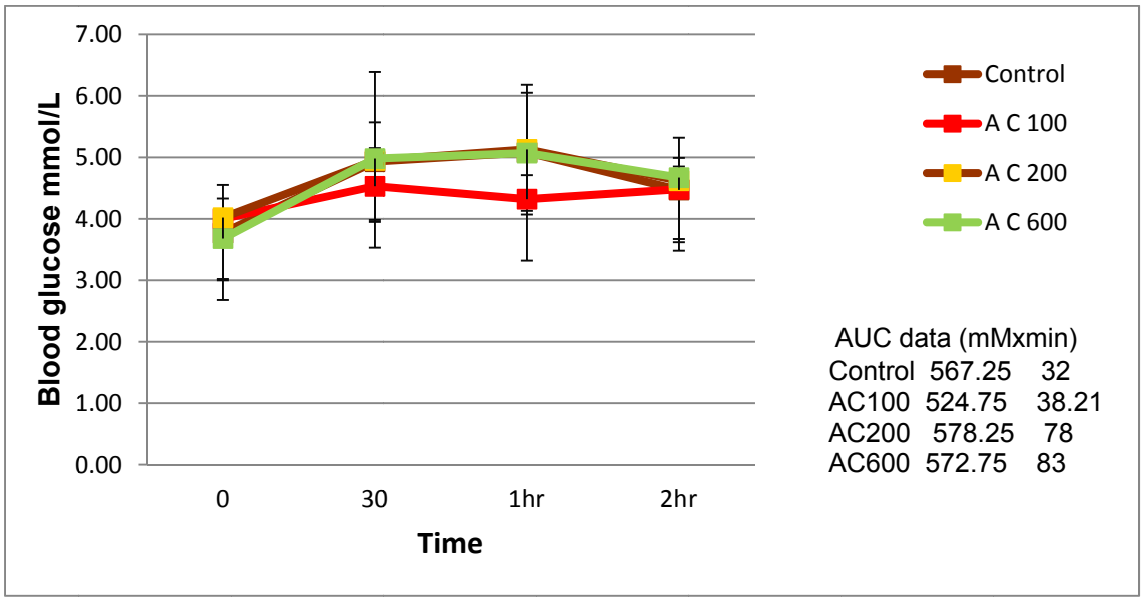

Figure B: High calorie fed

Figure 4.7.3 shows the glucose clearance rate in normal diet fed animal (A) and high calorie diet fed animals(B) in control, AC100, AC200 and AC600 groups

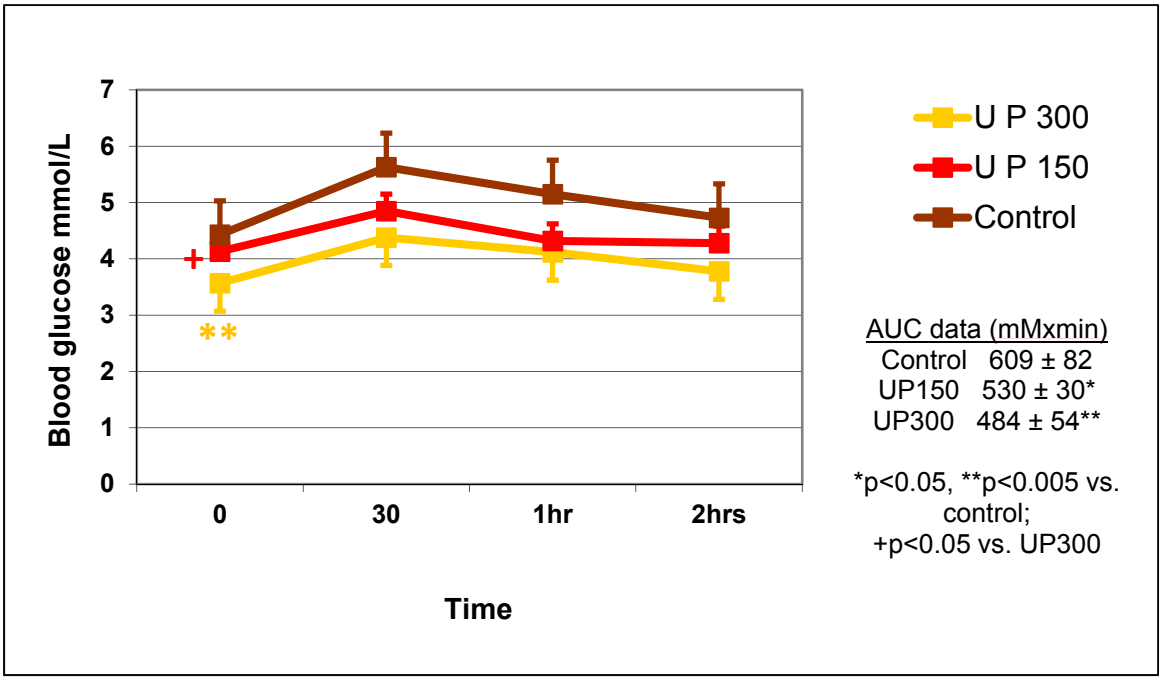

Figure A: Normal diet fed

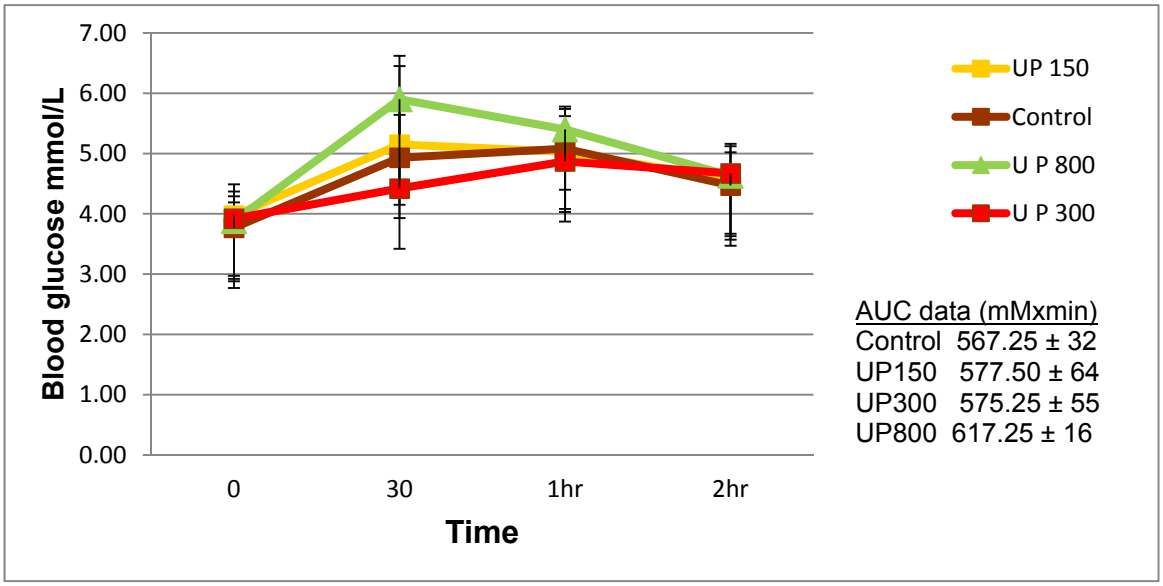

Figure B: High calorie fed

Figure 4.7.4 shows the glucose clearance rate in normal diet fed animal (A) and high calorie diet fed animals (B) in control, UP150, UP300 and UP800 groups

4.7 Description of the effect of plant extracts in animals during the OGTT.

Figures. 4.7.1 – 4.7.4describe the effects of the plant extracts on glucose concentration during an OGTT in both groups of animals. The results obtained from the OGTT showed that the extracts had a greater effect on glucose clearance in the animals fed on normal rat chow compared to the animals fed the high calorie diet (HCD).

Metformin and the mixed plant extracts had no effect on glucose concentration in either the normal diet fed or the high calorie diet fed groups of animals (Figure 4.7.1). This was also the case for all doses of *Icacina tricantha* (Figure 4.7.2). However, in the normal diet fed animals AC200 produced lower fasting glucose concentration and total (AUC) glucose concentration when compared to both control and AC100 animals (Figure 4.7.3). No effect of *Ananas cosmos* was seen in the high calorie diet animals. Figure 4.7.4 shows that in the normal diet fed animals, *Uraria picta*300 produced significantly lower fasting glucose concentration when compared to the control animals and those treated with *Uraria picta*150. Also, total (AUC) glucose concentration were significantly lower in the *Uraria picta*150 and *Uraria picta*300 treated animals than the control animals. No effect for the *Uraria picta* plant extract was observed in the high calorie diet fed animals.

CHAPTER 5 DISCUSSION

5.11 Selection of doses of plant extracts

The choice of doses for the plant extracts was made based on the literature and traditional herbal medicinal use of the plants (Igboechi and Osazuwa, 1988, 1989; Asuzu and Abubarkar, 1995; Asuzu and Ugwueze, 1990). Doses lower than the acute toxicity doses for the plant extracts were used. Also, the ratio for the formulation of the mixed plant extracts was chosen based on the ratio used by a herbalist. From traditional medicinal use, *Uraria picta* and *Ananas cosmos* are known to be quite safe while *I. trichantha*is known to have emetic effects. In fact *Uraria picta* is consumed as a vegetable by some cultures in Nigeria. The metformin dose of 300mg/kg was chosen based on several publications including that by Nobuyuki et al (2004). The plant extract doses used in this study are similar to the traditional medicinal doses except that in traditional herbal medicinal use, the patient is asked to take the aqueous extract three times daily. In this study, the plant extracts were administered only once daily.

5.12 Toxicity and safety studies

Before the commencement of the main studies and approval by the animal ethics committee, permission was given to conduct safety and toxicity studies to ascertain the safe doses of the plant extracts to be used in the studies. Two studies were conducted. In both studies, the highest possible doses of the extracts to be

administered were given to the animals daily for three weeks. In both studies, the extracts were found to be safe to be administered at those doses as no death was recorded and the animals did not show any adverse symptoms during the period of observation.

5.13 Effect of plant extracts on food intake and body weight

It has been established that feeding different species of animals on high calorie diets of varying formulations for different durations leads to insulin resistance but not diabetes (Chalkey et al, 2002). The severity of insulin resistance in animals is highly affected by the diet composition, duration of feeding and the animal species. Rats have been known to produce wide variations in fasting plasma glucose concentration with associated hyperglycaemia, (Han et al, 1997), variations in insulin concentration between the normal diet fed group and high calorie diet fed animals (Noshiro et al, 1997) and different responses in terms of weight gain.

Even though there is no perfect animal model that totally simulates the physiological and biochemical effects in humans of diabetes and obesity, most models are still able to provide valuable information (Cefalu, 2006).

Among out-bred Sprague Dawley rats fed on a high fat diet, “approximately one-half develop diet-induced obesity (DIO) and one-half are diet resistant (DR) on a diet relatively high in fat and energy content (HE diet)” gaining weight and fat at the same

rate as chow-fed controls however having increased fat pad, higher insulin and leptin concentration when compared to animals fed on normal rat chow (Levin et al, 1997, Levin and Keesey, 1998).

In the present study, the animals were fed on a high sucrose diet. The estimated percentage of sucrose in the diet is 18 %.

Kanazawa et al (2003) reported that feeding rats on a high sucrose (60% sucrose) diet for 2 weeks does not induce obesity in lean rats or enhance weight gain in obese rats. Santuré et al, 2002demonstrated that, after 4 weeks of feeding, rats displayed comparable final body weight regardless of whether they had been fed high sucrose or the normal chow diet. However, the average daily ad libitum intake was significantly lower for the sucrose-fed rats compared to their control chow-fed rats. Long term feeding of rats for up to 42 weeks on a high sucrose diet (63% sucrose) as reported by Fortino et al (2006), induced dyslipidemia, glucose intolerance, and adiposity.

Rats fed on a high sucrose diet were also observed to consume lesser amounts of food when compared to animals fed on the normal rat chow. (Fortino et al (2006)

In this study, the animals were fed for 16 weeks before dosing with the plant extracts commenced. The average weight of the animals fed on the high calorie diet was observed to be slightly lower than those of rats fed the normal rat chow before dosing

with the plant extracts. Despite this, the high calorie diet fed animals had higher insulin and glucose concentration than the normal diet fed animals before the plant extracts were administered to them, a result similar to that reported by other authors (Levin et al, 1997, Levin and Keesey, 1998). Also, weight gain is affected and determined by the diet composition and the duration of feeding. Lombardo et al (1996) showed that weight gain in rats fed on a rich sugar diet for the first 15 weeks did not differ between groups. A significant increase in weight gain was however observed in animals fed from 15 – 30 weeks. In the present study, the animals were fed for 16 weeks before dosing.

Meformin was observed to bring about a sustained decrease in the body weight of the animals in the normal diet fed group without affecting food intake. This correlates with the known effect of metformin to reduce weight in obese and diabetic rats (Bailey et al, 1996).Chronic administration of metformin (300mg/kg, subcutaneously administered) in obese Zucker rats, has been shown to significantly reduce food intake in rats whereas, 150mg/kg had no effect. In this experiment, 300mg/kg body weight of metformin was administered orally. The same group of researchers reported that intragastric administration of metformin did not have any effect on the food intake in rats. Data collated in this study correlates with these findings, as metformin was observed to bring about the sustained reduction of weight and oral dosage of metformin was observed not to affect food intake. (Rouru et al, 1995)
In the present study, the mixed plant extracts and *Uraria picta*150 caused greater weight gain in high calorie diet animals when compared to the control group. This

may be explained by higher food intake in these animals. However, in the normal diet fed groups, these plant extracts caused weight loss relative to the control groups, despite increased food intake relative to the control animals. These differential effects of the plant extracts in high calorie diet and normal diet fed animals are interesting and suggest that the extracts have effects on metabolism that are altered directly or indirectly by dietary intake. Energy expenditure was not measured in this study so we cannot make any conclusions on whether the weight effects of the plant extracts are mediated by effects on energy expenditure. The *Ananas cosmos* plant extract also caused weight loss relative to the control animals, but had no effect on food intake. Other studies have shown that medical plants decrease body weight in rats (Tian et al 2004) and the ability of some of the plant extracts to decrease body weight portends a possible functionality in the improvement of type II diabetes associated with obesity and weight gain (Bailey et al, 1996).

The average daily food intake in the animals in this study is comparable to that documented by Ace Animals Inc. (website reference 6), a breeder of Sprague Dawley rats in the United States. This is given as approximately 30grams/day.

5.14 Effect of plant extracts on glucose concentration

It has been established in a number of studies that feeding rats on a high sucrose concentration diet leads to glucose intolerance, a condition that predisposes to type II diabetes (Lombardo and Chicco, 2006). The present study also shows that, prior to

dosing with the plant extracts, glucose concentration were higher in the high calorie diet fed rats compared to the normal diet fed animals, despite the former group having a slightly lower mean body weight.

In this study, the effect of the different plant extracts on plasma glucose concentration in both normal fed and high calorie diet fed rats was evaluated. Plant extracts are known to elicit different responses in rat plasma glucose (Gallagheret al, 2003). Grover et al (2002) reviewed over 40 Indian medicinal plants that have shown both hypoglycemic and anti-hyperglycemic activity.Some plant extracts have been shown to act by abolishing the activities of glucose-6-phosphate dehydrogenase and 6-phosphate gluconate dehydrogenase in the intestinal mucosa-epithelial tissue (IMET) and liver (Netzer et al, 2003).

The metformin treatment in the normal diet fed group led to a significant reduction in glucose concentration in the last week when compared to the control group, whilst metformin in the high calorie diet group caused no significant change in glucose concentration although they did tend to be lower than the concentrations observed in the plasma of the control animals at week 1 and week 3. It is possible that the dose of metformin used in the high calorie diet group was too low to bring about a significant change in glucose concentration.

The effect of dosing with the different plant extracts on blood glucose varied in the different groups of animals. Some extracts did reduce blood glucose concentration

when compared to the control animals e.g. *Ananas cosmos*100 in the normal diet fed group but only at week 2 and *Uraria picta*800 in the high calorie diet animals, but only by week 3. Other extracts actually increased glucose concentration e.g. Ananas cosmos (AC) 100 and AC200 in the high calorie diet group and the mixed extracts at weeks 1 and 3 in the high calorie diet fed animals. However, these effects were small when compared to the changes observed in insulin concentration for animals treated with the plant extracts (see next section).

The fluctuation of the glucose concentration during the period of dosing may be an indication that the plant extracts contain glucose lowering substances, but not in sufficient quantities to maintain a sustained reduction in glucose concentration. Also, the possibility that in some animals, glucose concentration rose due to the massive reduction in insulin concentration caused by the plant extracts will be discussed in the next section.

A major reason why the plant extracts could not bring the glucose concentration under control may be due to inadequate dosages and the route of administration. Rouru et al, (1995) has demonstrated that chronic administration of metformin subcutaneously improves efficacy of the drug. Oral administration exposes the extracts to a first pass effect leading to lesser availability of the drug. Also, in traditional herbal medicinal use, divided doses of the plant extracts are taken three to four times every day while dosing was carried out once daily every morning in the rats. These factors could negatively impact on the potency of the extracts.

5.16 Effect of plant extract dosing on insulin and insulin sensitivity

The results clearly show that the plant extracts significantly improve insulin sensitivity and insulin concentration in both normal diet fed and high calorie diet fed animals. This effect was sustained in both the normal fed and high calorie diet groups of animals. When compared to the control group and baseline values, the ability of the plant extracts to bring down insulin concentration was observed to be the most prominent effect of the extracts. By default, the reduced insulin value also impacts on the HOMA value, which is used to determine insulin sensitivity.

A number of studies have established the induction of hyerinsulinemia and reduced insulin sensitivity in animals fed on high fat and high sucrose diets over a long period of time (Fortino et al, 2007). Data collated from this study is also in support of this trend. Basal insulin concentration and HOMA values in high calorie diet animals, before commencing treatment with the plant extracts, were observed to be five to seven times higher than those in the normal diet fed animals. These results are also supported by a number of studies where animals have been fed on high fat, high sucrose diets (Kawasaki et al 2005, Noshiro et al 1997).

In the control animals during the 3 week dosing period, insulin and HOMA concentration increased over time in the normal diet fed animals and this may represent the well-known effect of aging on insulin resistance. However, in the high calorie diet rodents, insulin concentration did not change over time and this may be partially explained by the fall in body weight observed in these animals over the

course of the experiment. Metformin treatment led to major reductions in insulin and HOMA concentration in the normal diet fed rats, whilst concentration were also lower in the high calorie diet animals, the effect was not significant. Metformin is a well-known insulin sensitiser, so these effects were not unexpected.

It is interesting to note that the effect of the plant extracts on insulin concentration was observed in the normal diet fed rats within the first week of administering the extracts whilst in the high calorie diet animals the effect was observed only after 2 weeks. This may be due to the higher baseline insulin and HOMA concentration observed in the high calorie diet animals. This delayed response to the plant extracts in the high calorie diet fed animals may explain the 2-way ANOVA results where significant interactions were observed between plant extract use and insulin and HOMA AUC levels.

The mixed plant extract did not have a significant effect on insulin concentration in the high calorie diet animals. It should also be noted that *Ananas cosmos (*AC)100 and AC200 caused increases in blood glucose in the high calorie diet animals. This is possibly not due to the massive effect on the attenuation of insulin concentration since AC100 increased glucose concentration at week 1 but there was no parallel reduction in insulin concentration. Also, at week 3 similar reductions were observed in insulin concentration for *Ananas cosmos (AC)*100 and AC200 yet only in the AC200 animals did glucose concentration rise significantly above control concentration.

Because plants contain a number of active constituents that are soluble in particular solvents and can be extracted using different solvents, different results may be expected depending on the extraction procedure used on the plants. Studies by Phuong et al, (2004) on the petroleum ether extract of *Nigella sativa* seeds on blood glucose, insulin and lipids in normal rats brought about a reduction of insulin but had no effects on glucose.

This is an indication that the plant extracts administered to the rats were able to improve the capacity of the cells to respond to insulin.In traditional herbal medicine the use of poly herbal medication is quite common place (Dixit et al, 1999,Sachidananda et al 2001).

5.17 Effect of plant extract dosing on free fatty acids.

The serum FFA concentration rose above baseline values in the control, normal diet fed animals, at week 1 but fell back to baseline by week 3. No changes in FFA concentration were observed in the high calorie diet animals. Metformin did reduce FFA concentration in the normal diet fed animals at week 1 but the effect was not sustained. Metformin would be expected to reduce FFA concentration as it is an insulin sensitising agent and would thus augment the ability of insulin to inhibit triglyceride lipolysis.

Apart from IT100 and *Ananas cosmos*200whichsignificantly brought down the level of FFAs, but only transiently, the effect of the other plant extracts were not significant.This demonstrates that the effects of the plant extracts on insulin sensitivity were not mediated through effects on serum FFAconcentration.

5.18 Effect of plant extract dosing on the OGTT.

The results in this study demonstrate the glucose lowering effect of some of the plant extracts. The administered doses of *Ananas cosmos* and *Uraria picta* in the normal diet fed animals significantly improved the glucose clearance rates. None of the different plant extracts had any significant effect on the glucose clearance rate in the high calorie diet fed animals.

While a number of medicinal plants have been shown to have hypoglycaemic effects and improve the glucose clearance rate in animals (Soo et al, 2009), studies such as those conducted by Al-Awadi FM. and Gumaa KA, (1986), investigating the antidiabetic effect of five Kuwaiti medicinal plants concluded that only two of the plant extracts showed glucose lowering effect using the OGTT. A similar pattern is seen in this study.

The OGTT data suggests that the major effect of some of the plant extracts i.e. *Icacina. trichantha* is to improve insulin sensitivity but not to alter glucose concentration, whilst for *Ananas cosmos* and *Uraria picta* insulin sensitivity is

improved in conjunction with improved postprandial glucose clearance. It is possible that these different effects on glucose concentration are the result of the greater efficacy of *Ananas cosmos* and *Uraria picta* to improve insulin sensitivity when compared to IT. The lack of effect of the plant extracts on the OGTT glucose concentration in the HCD fed animals may be due to the high level of insulin resistance at baseline in these animals, with the plant extracts not able to sufficiently reduce this to a level that leads to improved glucose clearance.

5.19 Clinical relevance of this study

The high calorie diet did not lead to obesity or type 2 diabetes but it did have profound effects on insulin concentrations and more modest effects on glucose concentrations, as described earlier in this chapter. Thus, we were unable to produce a model of type 2 diabetes using this diet. However, the animals fed the high calorie diet certainly expressed a phenotype of insulin resistance. All the plant extracts, with the exception of the mixed extract, were able to attenuate the insulin resistance observed in this group of animals. This is a very important finding and suggests that the plant extracts may have insulin sensitising effects which would be very useful for treating human type 2 diabetes in which insulin resistance is one of the prime aetiological factors. Obviously, the efficacy of these plants in human diabetic subjects would need to be tested in a clinical trial, however the fact that these plants are used for treating diabetes in African populations suggests there may be some clinical benefit. Whether these plant extracts are able to improve the insulin secretory dysfunction that is observed in human type 2 diabetes is not known and would

require further investigations in other more appropriate rodent or *in vitro* model systems.

The active ingredients of the extaracts that ameliorate insulin resistance need to be isolated and characterised. This may lead to the development of possible new therapies for diabetes, as the isolation of metformin from the French lilac so ably demonstrates.

5.20 Limitation of the study.

1. The present study was characterised by a number of limitations including the following:

2. The sample size of 6 rats per group is rather small. It is possible that this may have led to an inability to detect more subtle changes in a number of the investigated parameters, particularly food intake and glucose levels.

3. Even though rodents have been widely used and acknowledged as acceptable experimental animals, results and findings from studies like this cannot be transposed to humans.

4. The animals in this study did not develop type 2 diabetes and therefore we cannot show that the plant extracts ameliorate the metabolic dysfunction associated with this disorder. The use of diabetic Zucker rats or other similar rodent models of type 2

diabetes may clarify this. However, the animals fed the high calorie diet did develop profound insulin resistance, which is a key component of type 2 diabetes.

5. Better methods of extraction like freeze drying may have been employed to extract the active ingredients of the plants. The process employed in this research entailed drying the solution in an oven for days until dryness. This process may have denatured some of the active constituents of the plant extracts. Better methods of purification of the crude extracts could have also been employed to obtain a purer extract.

6. Working with plant extracts poses a lot of challenges peculiar to phyto-chemical research. It is an established fact that the pharmacological activities of plants are governed and affected by a number of factors including weather, soil composition, time and season of harvesting, method of storage, and processing and preservation. Even though the researcher endeavoured to secure plants from a particular seller at a particular month, these parameters may still have affected the efficacy of the plant extracts. Two different batches of plant extracts were used in this study and it is therefore possible that batch-to-batch variation may have affected some of the outcomes of this study.

7. The mechanisms by which these extracts reduce insulin sensitivity was not explored in the present study. Therefore, we cannot clearly state that the extracts

have direct effects on insulin sensitivity. It is possible that the extracts act indirectly or are metabolised to agents that effect insulin activity.

8. The plant extracts did not produce a clear dose response on some of the measured variables. The reasons for this are uncertain, but an understanding of their molecular modes of action may help to clarify this response.

9. The plant extracts were crude and the active ingredients are not known. Further work is required to purify the active ingredients and characterise them.

10. Measurements of variables analysed during the dosing period were not made during the 4 months when animals were being given the normal or high calorie diet. Measurements were made at baseline but data on body weight and food intake would have been useful to see how the 2 diets affected both these parameters in the run-in to the dosing experiments.

11. The measurement of HbA1c was not performed. This may have given some information on the level of glucose tolerance in each treatment group before the plant extracts were administered and would also have given useful information on the level of postprandial glycaemia during the dosing period.

5.21 Conclusions

The plants used in the present studies were observed to be safe for use in all the animals, even in large doses. This present study also suggests that the crude plant extracts may have value in modifying insulin resistance, a syndrome that predisposes to type II diabetes.

The stated aims of the research were achieved. The individual plant extracts in normal diet fed animals were observed to lower insulin concentration and improve insulin sensitivity. In the high calorie diet fed animals, a similar pattern of lowering the concentration of insulin plasma concentration and improving insulin sensitivity was observed. The combination of the plant extract was not as efficacious as the individual plant extracts. In comparison to metformin, some of the plant extracts were observed to have a greater insulin-sensitising effect.

The effect of the plant extracts on FFA concentrations and weight was minimal. From the data collated and analysed it can be seen that the plant extracts helped improve insulin resistance in the rats, suggesting that the plants may be used to prevent or improve insulin sensitivity in subjects predisposed to type 2 diabetes. However, these findings must be tested in human clinical trials.

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Appendix

Tables

Table 4.1.1 weight of normal diet fed rats during the period of dosing.

Group mg/kg	Week	Baseline	1	2	3
Control	(Rat code) 15a	682.00	702.50	692.00	693.00
	15b	595.00	597.50	597.50	590.00
	15c	550.00	535.50	535.50	537.00
	16a	531.00	548.00	548.00	548.00
	16b	602.00	615.50	603.00	605.00
	16c	700.00	703.00	680.00	691.00
	Mean ± SD	610.00 ± 68.43	617.00 ± 72.79	609.33 ± 65.15	610.67 ± 67.89
	Median (%)	100.00	101.34	100.29	99.83
	Quartile Range	0.00	2.59	4.10	2.90
Metformin	24a	597.00	575.00	567.00	552.00
	24b	534.00	514.00	494.00	489.00
	24c	640.00	623.00	607.00	595.00
	20a	631.00	613.00	601.00	567.00
	20b	615.00	582.00	568.00	567.00
	20c	586.00	566.00	550.00	537.00
	Mean ± SD	600.50 ± 38.33	578.83 ± 38.76	564.50 ± 40.84	551.17 ± 36.02
	Median (%)	100.00	96.45	94.35	91.92
	Quartile Range	0.00	0.89	2.47	0.09
Mixture	21a	680.00	650.00	635.00	672.00
	21b	547.00	550.00	541.00	571.00
	21c	725.00	726.00	724.00	739.00
	22a	722.00	723.00	698.00	717.00
	22b	619.00	616.50	573.00	620.00
	22c	612.00	596.00	579.00	609.00
	Mean ± SD	650.83 ± 70.31	643.58 ± 70.56	625.00 ± 73.63	654.67 ± 65.70
	Median (%)	100.00	99.87	95.64	99.84
	Quartile Range	0.00	2.75	5.50	2.62
I T 50	2a	622.50	624.00	617.50	615.50
	2b	573.50	570.50	568.50	565.00

	2c	629.00	622.50	623.00	615.50
	1a	639.00	655.00	646.50	641.00
	1b	640.00	646.00	643.00	647.00
	1c	592.00	606.00	599.50	598.00
	Mean ± SD	616.00 ± 27.20	620.67 ± 30.22	616.33 ± 29.11	613.67 ± 29.93
	Median (%)	100.00	100.59	99.83	99.59
	Quartile Range	0.00	2.89	2.10	2.50
I T 100	3a	625.00	631.00	615.00	604.00
	3b	676.00	677.00	670.00	666.00
	4a	632.00	642.00	633.00	630.00
	4b	627.00	645.00	633.50	627.50
	4c	660.00	658.00	654.50	655.00
	Mean ± SD	644.00 ± 22.77	650.60 ± 17.61	641.20 ± 21.33	636.50 ± 24.45
	Median (%)	100.00	100.96	99.17	99.24
	Quartile Range	0.00	1.43	1.05	1.16
A C 100	13a	652.00	650.00	642.00	631.00
	13b	705.00	702.00	692.50	695.00
	13c	625.00	622.00	615.00	604.50
	10a	667.00	667.00	663.00	660.50
	10b	601.00	596.00	586.00	582.50
	10c	570.00	567.00	556.50	559.50
	Mean ± SD	636.67 ± 8.33	634.00 ± 49.08	625.83 ± 50.20	622.17 ± 50.33
	Median (%)	100.00	99.55	98.13	97.54
	Quartile Range	0.00	0.21	0.84	1.80
A C 200	7a	669.00	671.00	672.00	669.00
	7b	715.00	702.50	698.00	695.00
	7c	598.00	607.00	596.00	600.00
	9a	619.00	626.50	622.50	622.50
	9b	616.00	610.00	606.00	607.50
	9c	685.00	679.00	670.00	670.00
	Mean ± SD	650.33 ± 46.11	649.33 ± 40.09	644.08 ± 41.44	644.00 ± 39.07
	Median (%)	100.00	99.71	99.01	99.31
	Quartile Range	0.00	2.19	2.64	2.52
U P 150	18a	743.00	718.00	702.00	715.00
	18b	692.00	653.00	634.50	648.00
	18c	634.00	598.00	576.00	586.00

	19a	656.00	628.50	607.50	614.00
	19b	692.00	668.00	652.00	661.00
	19c	684.00	652.00	640.00	637.00
	Mean ± SD	683.50 ± 37.13	652.92 ± 40.19	635.33 ± 42.52	643.50 ± 43.95
	Median (%)	100.00	95.57	93.09	93.62
	Quartile Range	0.00	2.17	2.52	2.39
U P 300	6a	594.00	594.00	579.00	570.00
	6b	724.00	701.00	707.00	716.00
	6c	692.00	675.00	666.50	650.00
	11a	695.00	677.50	662.00	671.00
	11b	770.00	726.50	669.00	717.00
	11c	705.00	675.00	718.00	678.00
	Mean ± SD	696.67 ± 57.90	674.83 ± 44.49	666.92 ± 48.94	667.00 ± 54.29
	Median (%)	100	97.15	96.89	96.07
	Quartile Range	0	1.8	2.4	2.62

Table 4.1.2 weight of high calorie diet fed rats during the period of dosing

Group mg/kg	Week	Baseline	1	2	3
Control	1a	656	640	615	605
	1b	565	554	540	535
	1c	567	560	550	556
	2a	750	770	720	701
	2b	648	633	603	595
	2c	703	706	663	655
	Mean ± SD	648.20 ± 73.41	643.83 ± 83.68	615.20 ± 68.30	607.83 ± 61.76
	Median (%)	100.00	98.40	94.94	93.31
	Quartile Range	0.00	2.74	2.25	2.47
Metformin	3a	652	638	607	605
	3b	725	720	694	690
	3c	564	540	526	530
	4a	628	531	508	510
	4b	562	560	582	570
	4c	620	618	582	580
	Mean ± SD	625.20 ± 60.75	601.20 ± 72.28	583.20 ± 65.99	580.83 ± 63.59
	Median (%)	100.00	98.58	93.57	93.76

	Quartile Range	0.00	3.90	2.63	2.38
Mixture	5a	642	632	630	625
	5b	662	648	640	633
	5c	578	564	560	557
	6a	562	578	556	554
	6b	656	656	644	640
	6c	672	672	666	659
	Mean ± SD	628.67 ± 46.74	625.00 ± 44.00	616.00 ± 46.46	611.33 ± 44.70
	Median (%)	100.00	99.22	98.15	97.46
	Quartile Range	0.00	2.11	2.04	1.70
I T 50	7a	610	606	588	579
	7b	670	610	642	630
	7c	666	666	634	629
	8a	626	630	598	591
	8b	652	662	620	610
	8c	680	702	674	669
	Mean ± SD	650.67 ± 27.33	646.00 ± 37.27	626.00 ± 31.27	618.00 ± 32.19
	Median (%)	100.00	100.31	95.67	94.43
	Quartile Range	0.00	2.19	1.20	0.89
I T 100	9a	654	654	630	625
	9b	620	610	592	595
	9c	540	532	514	507
	10a	510	498	480	495
	10b	706	694	678	667
	10c	624	624	606	598
	Mean ± SD	609.00 ± 72.58	602.00 ± 74.07	583.33 ± 73.77	581.17 ± 67.36
	Median (%)	100.00	98.45	95.76	95.70
	Quartile Range	0.00	1.70	1.15	1.49
A C 100	11a	540	542	528	525
	11b	554	550	520	517
	11c	480	470	458	460
	12a	640	620	612	615
	12b	660	660	638	630
	12c	680	692	668	658
	Mean ± SD	592.33 ±	589.00 ±	570.67 ±	567.50 ±

		79.20	83.07	80.99	77.80
	Median (%)	100.00	99.64	96.16	95.96
	Median	0.00	2.45	2.36	1.30
A C 200	13a	580	580	582	580
	13b	590	590	590	595
	13c	690	692	670	665
	14a	590	570	546	545
	14b	664	668	664	665
	14c	560	560	542	545
	Mean ± SD	612.33 ± 51.93	610.00 ± 55.66	599.00 ± 56.02	599.17 ± 54.63
	Median (%)	100.00	100.00	98.55	98.66
	Quartile Range	0.00	0.09	3.21	3.77
AC 600	15a	687	682	668	652
	15b	724	735	707	695
	15c	584	560	537	541
	16a	600	591	562	549
	16b	625	618	604	595
	16c	628	585	578	565
	Mean ± SD	641.33 ± 53.59	628.50 ± 66.79	609.33 ± 65.52	599.50 ± 61.74
	Median (%)	100.00	98.69	95.15	93.77
	Quartile Range	0.00	3.38	5.20	3.70
U P 150	17a	680	676	670	675
	17b	614	606	590	600
	17c	600	598	580	565
	18a	562	576	558	560
	18b	670	676	652	650
	18c	548	536	526	530
	Mean ± SD	612.33 ± 54.28	611.33 ± 55.67	596.00 ± 55.22	596.67 ± 56.18
	Median (%)	100.00	99.54	96.99	97.37
	Quartile Range	0.00	2.19	2.44	2.55
U P 300	19a	624	612	588	580
	19b	624	612	578	575
	19c	590	570	558	560
	20a	574	564	552	545
	20b	628	624	598	600
	20c	600	640	562	565

	Mean ± SD	606.67 ± 22.11	603.67 ± 30.26	572.67 ± 18.23	570.83 ± 18.82
	Median (%)	100.00	98.17	94.40	94.54
	Quartile Range	0.00	1.29	1.56	2.00
UP 800	21a	690	647	604	595
	22b	570	552	524	519
	22c	588	587	582	579
	23a	655	635	630	632
	23b	575	567	553	549
	23c	652	650	619	620
	Mean ± SD	621.67 ± 50.36	606.33 ± 43.02	585.33 ± 40.73	582.33 ± 42.85
	Median (%)	100.00	97.78	95.56	95.29
	Quartile Range	0.00	2.85	4.25	5.44

Table 4.2.1 weight of feed (g) consumed in normal diet fed animals.

	Animals per cage	1st week	2nd week	3rd week
Control	3	23.56	22.39	20.56
	3	24.50	23.80	21.40
	Mean	**24.03**	**23.10**	**20.98**
	STD	0.6646804	0.9970206	0.59397
Metformin	3	29.10	25.80	25.30
	3	28.60	26.70	26.10
	Mean	**28.85**	**26.25**	**25.70**
	STD	0.3535534	0.6363961	0.565685
Mixture	3	26.30	30.10	27.31
	3	27.50	30.10	22.00
	Mean	**26.90**	**30.10**	**24.66**
	STD	0.8485281	0	3.754737
I T 50	3	22.83	23.17	26.35
	3	23.83	30.94	28.00
	Mean	**23.33**	**27.06**	**27.18**
	STD	0.7071068	5.4942197	1.166726
I T 100	3	23.04	22.22	29.98
	3	25.40	23.61	28.71
	Mean	**24.22**	**22.92**	**29.35**
	STD	1.668772	0.9828784	0.898026
A C 100	3	25.67	24.39	20.44
	3	25.22	25.44	21.00
	Mean	**25.45**	**24.92**	**20.72**
	STD	0.3181981	0.7424621	0.39598
A C 200	3	25.21	22.83	30.60
	3	23.90	22.67	21.67
	Mean	**24.56**	**22.75**	**26.14**
	STD	0.9263099	0.1131371	6.314464
U P 150	3	29.60	31.40	30.30

	3	29.70	30.50	29.40
	Mean	**29.65**	**30.95**	**29.85**
	STD	0.0707107	0.6363961	0.636396
U P 300	**3**	27.10	31.50	31.10
	3	30.10	31.80	28.20
	Mean	**28.60**	**31.65**	**29.65**
	STD	2.1213203	0.212132	2.05061

Table 4.2.2 weight of feed consumed in diet fed animals.

	Animals per cage	1st week	2nd week	3rd week
Control	2	29.25	36.50	37.12
	2	35.50	33.50	41.38
	2	30.50	35.00	38.00
	Mean	31.75	35.00	38.83
	STD	2.70	1.22	1.84
Metformin	2	28.70	22.30	30.80
	2	26.70	30.10	31.50
	2	33.00	22.40	23.40
	Mean	29.47	24.93	28.57
	STD	3.22	4.47	4.49
Mixture	2	25.30	31.00	34.70
	2	21.30	32.30	33.30
	2	20.30	23.00	30.90
	Mean	22.30	28.77	32.97
	STD	2.65	5.04	1.92
I T 50	2	31.30	30.70	25.00
	2	40.60	25.00	27.60
	2	39.30	29.50	30.40
	Mean	37.07	28.40	27.67
	STD	5.04	3.00	2.70

I T 100	2	39.00	25.30	27.00
	2	28.10	28.30	37.00
	2	28.00	22.00	24.20
	Mean	31.70	25.20	29.40
	STD	6.32	3.15	6.73
A C 100	2	24.30	26.75	32.50
	2	28.30	24.80	30.90
	2	21.70	27.50	33.00
	Mean	24.77	26.35	32.13
	STD	3.32	1.39	1.10
A C 200	2	26.30	22.80	41.00
	2	27.30	27.80	39.00
	2	24.30	30.00	38.70
	Mean	25.97	26.87	39.57
	STD	1.53	3.69	1.25
AC 600	2	30.80	43.40	41.25
	2	27.80	38.10	37.63
	2	31.60	38.80	38.25
	Mean	30.07	40.10	39.04
	STD	2.00	2.88	1.94
U P 150	2	22.00	25.00	30.90
	2	24.30	23.00	43.00
	2	28.00	29.80	43.80
	Mean	24.77	25.93	39.23
	STD	3.03	3.49	7.23
U P 300	2	21.50	34.00	27.20
	2	21.30	26.30	31.20
	2	23.70	27.30	33.90
	Mean	22.17	29.20	30.77
	STD	1.33	4.19	3.37
UP 800	2	38.50	38.87	38.25
	2	32.50	39.50	36.00
	2	27.75	38.00	35.88
	Mean	32.92	38.79	36.71
	STD	5.39	0.75	1.34

Table 4.3.1: oral glucose tolerance test results in normal diet fed group.

Time (mins)		0	30	60	120
Group mg/kg	Animal code				
Control	15a	4.40	4.80	4.80	4.20
	15b	4.20	5.60	5.50	5.10
	15c	5.20	6.30	5.80	5.30
	16a	4.50	6.10	6.10	5.20
	16b	4.50	6.40	5.30	4.40
	16c	3.80	4.60	3.40	4.20
	Mean ±SD	4.43 ± 0.46	5.63 ± 0.78	5.15 ± 0.97	4.73 ± 0.52
Metformin	24a	4.70	5.60	4.30	4.10
	24b	3.30	4.80	4.40	4.20
	24c	4.50	5.40	4.70	3.30
	20a	4.10	4.60	4.60	4.70
	20b	3.80	5.80	5.40	4.10
	20c	4.70	5.50	4.90	4.30
	Mean	4.18 ± 0.56	5.28 ± 0.48	4.72 ± 0.40	4.12 ± 0.46
Mixed	21a	4.30	5.80	5.20	4.80
	21b	4.60	4.40	3.40	2.90
	21c	4.10	5.50	5.40	4.50
	22a	4.60	5.90	5.60	4.40
	22b	4.10	4.40	4.70	4.90
	22c	4.00	4.70	4.30	4.70
		4.28 ± 0.26	5.12 ± 0.70	4.77 ± 0.82	4.37 ± 0.74
I T 50	2a	3.70	5.70	4.50	4.20
	2b	3.80	4.60	5.30	3.60
	2c	3.10	3.30	3.90	3.20
	1a	4.40	6.40	5.40	4.30
	1b	3.80	6.50	5.80	4.20
	1c	3.80	4.90	4.90	4.10
I T 100	3a	4.80	5.50	5.80	5.40
	3b	4.30	5.70	5.40	4.90
	4a	3.90	7.30	4.20	4.10
	4b	3.70	3.80	4.10	3.60

	4c	6.60	4.20	5.40	4.40
		4.63 ± 1.16	5.35 ± 1.38	5.03 ± 0.78	4.48 ± 0.70
A C 100	13a	4.10	6.30	6.00	4.60
	13b	3.80	8.20	6.00	5.00
	13c	4.60	5.30	5.60	5.00
	10a	4.70	6.10	5.90	5.00
	10b	4.60	6.00	5.40	4.40
	10c	4.80	6.00	5.00	5.20
		4.43 ± 0.39	6.36 ± 0.98	5.65 ± 0.40	4.87 ± 0.30
A C 200	7a	3.60	4.50	4.20	3.20
	7b	3.70	4.70	3.20	3.50
	7c	3.80	4.40	3.80	4.70
	9a	3.50	4.70	5.50	4.80
	9b	4.30	4.70	4.60	4.70
	9c	4.30	4.80	5.30	4.80
	Mean	3.87 ± 0.35	4.63 ± 0.15	4.43 ± 0.88	4.28 ± 0.73
U P 150	18a	3.90	4.80	4.20	3.90
	18b	3.90	4.60	3.90	4.60
	18c	4.30	4.50	3.80	4.10
	19a	4.40	4.70	4.90	4.60
	19b	4.20	5.00	4.50	4.30
	19c	4.10	5.50	4.60	4.20
	Mean	4.13 ± 0.21	4.85 ± 0.36	4.32 ± 0.43	4.28 ± 0.28
U. P 300	6a	4.40	5.20	4.90	4.40
	6b	3.40	4.20	4.20	3.70
	6c	3.50	4.70	4.00	4.10
	11a	3.40	4.20	4.60	3.80
	11b	3.50	3.60	3.70	3.30
	11c	3.20	4.40	3.30	3.40
	Mean	3.57 ± 0.42	4.38 ± 0.54	4.12 ± 0.58	3.78 ± 0.42

Table 4.3.2: oral glucose tolerance test results in high calorie diet fed group.

Time (min)		0.00	30.00	60.00	120.00
	Rat code				
Control	1a	4.10	4.30	5.30	4.80
	1b	3.30	4.70	5.10	4.60
	1c	3.40	5.20	5.30	4.30
	2a	4.30	6.20	5.40	4.30
	2b	4.00	4.30	4.70	4.40
	2c	3.50	4.90	4.70	4.40
		3.77 ± 0.42	**4.93 ± 0.71**	**5.08 ± 0.31**	**4.47 ± 0.20**
Metformin	3a	4.20	4.50	4.10	4.10
	3b	3.60	5.70	5.20	3.70
	3c	3.00	5.50	4.90	3.50
	4a	3.40	5.00	5.20	4.40
	4b	3.80	5.80	5.70	4.90
	4c	3.60	5.30	5.02	4.12
		3.60 ± 0.40	**5.30 ± 0.49**	**5.02 ± 0.53**	**4.12 ± 0.53**
Mixture	5a	3.30	4.10	4.40	3.90
	5b	3.60	3.90	3.80	3.90
	5c	4.10	4.70	5.00	4.80
	6a	4.20	5.30	5.00	4.50
	6b	4.40	3.60	4.00	3.90
	6c	4.10	5.00	5.20	4.60
		3.95 ± 0.41	**4.43 ± 0.67**	**4.57 ± 0.59**	**4.27 ± 0.41**
I T 50	7a	3.80	5.00	4.90	4.90
	7b	4.70	5.80	4.90	4.90
	7c	5.40	5.30	4.90	5.00
	8a	4.20	3.90	4.20	4.90
	8b	4.50	5.60	5.40	5.40
	8c	4.30	5.90	5.60	5.10
		4.48 ± 0.54	**5.25 ± 0.74**	**4.98 ± 0.49**	**5.03 ± 0.20**

I T 100	9a	4.10	4.60	4.40	4.40
	9b	3.80	4.80	4.10	4.40
	9c	4.10	4.30	4.90	4.30
	10a	5.00	5.70	5.00	5.00
	10b	4.30	5.30	4.10	4.00
	10c	4.40	5.10	5.50	5.10
		4.28 ± 0.41	**4.97 ± 0.50**	**4.67 ± 0.56**	**4.53 ± 0.43**
A C 100	11a	4.20	3.40	3.60	4.30
	11b	4.20	4.60	4.00	5.00
	11c	3.60	4.90	4.60	4.40
	12a	4.40	4.20	4.40	4.90
	12b	4.10	4.30	4.60	3.90
	12c	3.50	5.80	4.70	4.40
		4.00 ± 0.33	**4.53 ± 0.62**	**4.32 ± 0.39**	**4.48 ± 0.37**
AC 200	13a	3.30	4.00	3.20	4.00
	13b	3.90	5.20	5.40	4.70
	13c	4.00	4.70	5.20	4.90
	14a	3.70	5.30	5.10	5.00
	14b	4.40	5.80	6.40	4.70
	14c	4.80	4.70	5.50	4.40
		4.02 ± 0.53	**4.95 ± 0.62**	**5.13 ± 1.05**	**4.62 ± 0.37**
AC 600	15a	3.20	4.70	4.30	4.00
	15b	3.60	5.00	5.40	4.70
	15c	3.80	5.20	4.20	4.60
	16a	4.30	5.70	5.80	5.40
	16b	3.90	5.40	6.50	5.40
	16c	3.30	3.90	4.20	3.90
		3.68 ± 0.41	**4.98 ± 1.41**	**5.07 ± 0.98**	**4.67 ± 0.65**
UP 150	17a	4.40	5.90	5.80	5.30
	17b	3.60	4.80	5.10	4.60
	17c	3.10	4.60	3.70	3.90
	18a	4.20	5.30	5.00	4.40
	18b	4.40	5.70	5.20	4.70

	18c	4.10	4.60	5.40	4.50
		3.97 ± 0.52	**5.15 ± 0.57**	**5.03 ± 0.71**	**4.57 ± 0.45**
UP 300	19a	4.30	5.10	5.80	5.20
	19b	3.60	5.90	5.50	4.70
	19c	3.80	5.00	4.30	5.00
	20a	4.20	5.70	5.30	4.80
	20b	4.20	4.80	4.20	4.00
	20c	3.40	5.00	4.10	4.30
		3.92 ± 0.37	**4.42 ± 2.20**	**4.87 ± 0.75**	**4.67 ± 0.45**
U P 800	21a	4.10	6.00	5.50	5.30
	22b	4.40	5.70	5.30	4.70
	22c	3.60	5.10	6.10	4.30
	23a	3.80	6.00	5.00	5.20
	23b	4.30	5.80	5.20	4.30
	23c	3.10	6.80	5.30	4.00
		3.88 ± 0.49	**5.90 ± 0.55**	**5.40 ± 0.38**	**4.63 ± 0.53**

Table 4.4.1 glucose concentration results in mmol/l for normal diet fed group.

Week	Week	1	2	3	4
	(Rat code)				
Control	15a	7.86	7.86	6.29	11.01
	15b	6.29	7.86	8.65	9.43
	15c	7.07	10.22	5.50	9.43
	16a	6.29	11.01	10.22	9.43
	16b	7.86	5.50	10.22	7.07
	16c	7.86	8.65	10.22	10.22
	Mean ± SD	7.21 ± 0.77	8.52 ± 1.95	8.52 ± 2.13	9.43 ± 1.32
	Median (%)	100	117.5	130	136.67
	Quartile Range	0	44.44	54.5	20
Metformin	24a	7.07	6.29	6.29	4.72
	24b	4.72	8.65	6.29	5.50
	24c	5.50	5.50	7.07	7.07
	20a	6.29	8.65	6.29	4.72
	20b	6.29	6.29	4.72	4.72
	20c	5.50	6.29	6.29	3.93
	Mean ± SD	5.90 ± 0.88	6.94 ± 1.35	6.16 ± 0.77	5.11 ± 1.08
	Median (%)	100	107.14	107.14	75
	Quartile Range	0	37.5	39.68	45.24
Mixture	21a	6.29	4.72	6.29	6.29
	21b	5.50	3.93	6.29	7.07
	21c	6.29	7.07	6.29	8.65
	22a	5.50	5.50	5.50	7.07
	22b	5.50	5.50	3.93	9.43
	22c	6.29	6.29	9.43	6.29
	Mean ± SD	5.9 ± 0.43	5.50 ± 1.11	6.29 ± 1.79	7.47 ± 1.29
	Median (%)	100	100	100	128.57
	Quartile Range	0	25	14.9	37.5
I T 50	2a	5.50	7.07	5.50	8.65
	2b	7.07	8.65	4.72	8.65
	2c	6.29	10.22	6.29	10.22
	1a	6.29	7.86	6.29	11.01
	1b	7.07	5.50	5.50	11.01
	1c	9.43	7.86	4.72	10.22

	Mean ± SD	8.38 ± 1.35	7.86 ± 1.57	5.50 ± 0.70	9.96 ± 1.07
	Median (%)	100	123.61	88.89	156.35
	Quartile Range	0	45.24	33.33	40.28
I T 100	3a	7.07	3.93	7.86	7.86
	3b	6.29	8.65	6.29	7.86
	4a	5.50	4.72	6.29	7.07
	4b	5.50	4.72	7.86	7.86
	4c	4.72	4.72	6.29	7.07
	Mean ± SD	5.82 ± 0.90	5.35 ± 1.88	6.92 ± 0.86	7.55 ± 0.43
	Median (%)	100	85.71	114.29	128.57
	Quartile Range	0	14.29	22.22	17.86
A C 100	13a	7.86	4.72	4.72	7.86
	13b	7.075	7.861	4.716	5.503
	13c	7.075	4.716	4.716	10.219
	10a	6.29	5.50	7.86	6.29
	10b	7.07	7.86	4.72	10.22
	10c	7.07	7.07	4.72	5.50
	Mean ± SD	7.07 ± 0.50	6.29 ± 1.49	5.24 ± 1.28	7.60 ± 2.20
	Median (%)	100	93.75	66.67	100
	Quartile Range	0	44.44	0	66.67
A C 200	7a	4.72	4.72	6.29	10.22
	7b	7.07	8.65	3.93	10.22
	7c	7.86	7.86	4.72	9.43
	9a	6.29	6.29	3.93	11.79
	9b	5.50	10.22	4.72	11.01
	9c	7.07	5.50	6.29	10.22
	Mean ± SD	7.6 ± 1.16	7.21 ± 2.07	4.98 ± 1.07	11.66 ± 0.81
	Median (%)	100	100	74.11	165.97
	Quartile Range	0	22.22	28.89	55.56
U P 150	18a	7.07	6.29	7.07	7.86
	18b	7.07	5.50	6.29	6.29
	18c	5.50	5.50	5.50	7.86
	19a	4.72	5.50	6.29	7.07
	19b	5.50	4.72	6.29	8.65
	19c	7.86	4.72	7.86	7.07
	Mean ± SD	6.29 ± 1.22	5.37 ± 0.59	14.54 ± 0.81	7.73 ± 0.82

	Median (%)	100	87.3	100	126.98
	Quartile Range	0	22.22	14.29	60
U P 300	6a	5.50	4.72	5.50	8.65
	6b	3.93	5.50	7.86	6.29
	6c	6.29	5.50	7.07	5.50
	11a	4.72	5.50	6.29	5.50
	11b	3.93	7.86	5.50	6.29
	11c	6.29	5.50	8.65	10.22
	Mean ± SD	5.11 ± 1.08	5.76 ± 1.07	6.81 ± 1.28	7.07 ± 1.93
	Median (%)	100	102.08	135.42	158.57
	Quartile Range	0	52.5	27.5	43.33

Table 4.4.2 glucose concentration in mmol/l results for high calorie diet fed group.

		Baseline	1	2	3
	Rat code				
Control	1a	8.41	9.18	7.65	9.18
	1b	9.94	8.41	21.41	9.18
	1c	9.94	9.18	13.00	10.71
	2a	9.18	10.71	9.18	8.41
	2b	9.18	8.41	9.18	10.71
	2c	7.65	9.18	16.82	6.12
	Mean±SD	9.05 ± 0.89	9.18 ± 0.84	12.87 ± 5.35	9.05 ± 1.75
	Median (%)	100.00	100.70	115.39	100.00
	Quartile Range	0.00	25.00	115.39	17.42
Metformin	3a	13.00	8.41	9.94	7.65
	3b	9.18	6.12	13.00	5.35
	3c	9.18	6.88	12.24	8.41
	4a	10.71	7.65	10.71	7.65
	4b	6.88	9.94	6.88	9.18
	4c	9.18	9.94	13.00	6.12
	Mean±SD	9.96 ± 2.09	8.38 ± 1.62	11.27 ± 2.42	7.60 ± 1.46
	Median (%)	100.00	73.21	116.67	69.05
	Quartile Range	0.00	41.67	41.67	32.84
Mixture	5a	4.72	8.65	9.43	7.07
	5b	4.72	7.07	7.07	6.29

	5c	4.72	7.86	9.43	6.29
	6a	3.93	9.43	9.43	6.29
	6b	5.50	7.86	11.79	7.86
	6c	6.29	9.43	7.86	9.43
	Mean±SD	4.98 ± 0.81	8.38 ± 0.95	9.17 ± 1.62	7.21 ± 1.26
	Median (%)	100.00	158.33	200.00	146.43
	Quartile Range	0.00	33.33	64.29	16.67
I T 50	7a	5.50	9.43	11.01	11.79
	7b	7.86	5.50	5.50	7.07
	7c	7.07	5.50	12.58	4.72
	8a	4.72	7.86	13.36	6.29
	8b	7.07	6.29	11.79	3.93
	8c	6.29	7.07	7.07	5.50
	Mean±SD	6.42 ± 1.16	6.94 ± 1.53	10.22 ± 3.18	6.55 ± 2.80
	Median (%)	100.00	100.69	172.22	88.75
	Quartile Range	0.00	88.89	87.50	66.67
I T 100	9a	3.93	6.29	6.29	4.72
	9b	6.29	5.50	9.43	14.15
	9c	4.72	7.07	12.58	6.29
	10a	7.07	6.29	11.79	5.50
	10b	5.50	7.86	6.29	4.72
	10c	7.86	7.86	11.01	14.15
	Mean±SD	5.90 ± 1.47	6.81 ± 0.95	9.56 ± 2.74	8.25 ± 4.60
	Median (%)	100.00	121.49	155.00	126.67
	Quartile Range	0.00	61.11	26.67	94.29
A C 100	11a	5.50	7.86	8.65	5.50
	11b	5.50	8.65	10.22	6.29
	11c	7.07	7.07	11.01	5.50
	12a	5.50	8.65	7.07	7.07
	12b	4.72	10.22	14.94	6.29
	12c	7.07	8.65	21.22	5.50
	Mean±SD	5.90 ± 0.96	8.53 ± 1.04	12.18 ± 5.16	6.03 ± 0.64
	Median (%)	100.00	150.00	171.43	107.14
	Median	0.00	34.92	144.44	50.79

AC 200	13a	5.50	7.86	7.86	8.65
	13b	4.72	7.07	6.29	6.29
	13c	5.50	9.43	6.29	18.08
	14a	5.50	7.86	5.50	13.36
	14b	5.50	10.22	4.72	12.58
	14c	5.50	9.43	7.86	11.79
	Mean±SD	5.37 ± 0.32	8.62 ± 1.22	6.42 ± 1.26	11.79 ± 4.07
	Median (%)	100.00	160.71	123.81	221.43
	Quartile Range	0.00	28.57	42.86	85.72
AC 600	15a	7.07	7.07	13.36	7.07
	15b	7.86	7.07	10.22	7.86
	15c	7.86	8.65	11.01	8.65
	16a	10.22	7.86	14.15	7.07
	16b	12.58	8.65	11.79	5.50
	16c	9.43	7.07	11.01	6.29
	Mean±SD	9.17 ± 2.03	7.73 ± 0.77	11.92 ± 1.53	7.07 ± 1.11
	Median (%)	100.00	83.46	134.23	84.62
	Quartile Range	0.00	25.00	23.33	33.33
UP 150	17a	7.07	6.29	8.65	3.93
	17b	6.29	17.29	7.07	11.01
	17c	5.50	7.07	14.15	5.50
	18a	5.50	4.72	8.65	7.07
	18b	5.50	8.65	13.36	6.29
	18c	6.29	7.07	10.22	7.86
	Mean±SD	6.03 ± 0.64	8.52 ± 4.49	10.35 ± 2.83	6.94 ± 2.41
	Median (%)	100.00	120.40	159.82	119.64
	Quartile Range	0.00	68.25	120.64	28.57
UP 300	19a	6.29	8.65	14.94	7.86
	19b	6.29	7.86	6.29	7.07
	19c	5.50	5.50	6.29	5.50
	20a	5.50	4.72	8.65	7.07
	20b	6.29	6.29	7.86	5.50
	20c	6.29	7.07	11.01	8.65
	Mean±SD	6.06 ± 0.41	6.68 ± 1.47	9.17 ± 0.32	6.94 ± 1.26
	Median (%)	100.00	106.25	141.07	118.75

	Quartile Range	0.00	25.00	60.17	28.57
UP 800	21a	7.07	7.86	12.58	6.29
	22b	8.65	11.79	13.36	9.43
	22c	14.94	11.01	12.58	7.86
	23a	11.01	9.43	13.36	7.86
	23b	10.22	8.65	12.58	3.14
	23c	10.22	8.65	15.72	6.29
	Mean±SD	10.35 ± 2.65	9.56 ± 1.53	13.36 ± 1.22	6.81 ± 2.15
	Median (%)	10.22	9.04	12.97	7.08
	Quartile Range	2.36	2.36	0.79	1.57

Table 4.5.1: calculated insulin concentration results in µu/ml for normal diet fed group.

Week	Week	Baseline	1.00	3.00	4.00
	(Rat code)				
Control	15a	6.51	8.02	11.27	12.21
	15b	7.27	6.51	14.69	10.56
	15c	4.00	7.27	8.02	9.74
	16a	5.63	10.56	8.02	8.92
	16b	5.63	12.21	9.77	13.04
	16c	4.82	9.74	14.78	12.21
	Mean ± SD	5.65 ± 1.17	9.05 ± 2.16	11.09 ± 3.08	11.12 ± 1.62
	Median (%)	100.00	184.69	184.00	209.47
	Quartile Range	0.00	79.16	28.94	85.29
Metformin	24a	5.51	0.75	0.75	1.00
	24b	1.55	0.75	1.00	2.37
	24c	4.01	1.00	4.00	4.00
	20a	4.76	0.75	1.00	2.37
	20b	5.51	0.75	4.76	6.45
	20c	3.18	2.37	4.01	3.18
	Mean ± SD	4.09 ± 1.53	1.06 ± 0.65	2.59 ± 1.85	3.23 ± 1.87
	Median (%)	100.00	20.40	75.41	99.88
	Quartile Range	0.00	34.71	78.71	67.33
Mixture	21a	7.27	1.00	0.75	0.75
	21b	5.63	1.00	3.26	0.75
	21c	3.18	0.75	0.75	0.75
	22a	7.27	6.51	1.50	0.75

	22b	4.82	1.50	0.75	0.75
	22c	4.00	0.75	0.75	0.75
	Mean ± SD	5.36 ± 1.69	1.92 ± 2.27	1.29 ± 1.01	0.75 ± 0.00
	Median (%)	100.00	21.20	19.73	14.47
	Quartile Range	0.00	13.43	8.00	8.46
I T 50	2a	4.00	0.74	1.55	3.26
	2b	2.37	1.55	2.37	0.74
	2c	5.63	0.74	0.65	1.50
	1a	4.00	0.74	0.74	0.69
	1b	5.63	0.74	0.74	0.40
	1c	3.26	1.55	0.74	3.18
	Mean ± SD	4.15 ± 1.30	1.01 ± 0.42	1.13 ± 0.69	3.26 ± 1.29
	Median (%)	100.00	18.57	20.64	29.01
	Quartile Range	0.00	34.56	25.72	64.15
I T 100	3a	5.63	2.00	0.75	3.18
	3b	6.45	0.75	0.75	0.75
	4a	4.00	0.75	0.75	0.75
	4b	6.45	0.75	5.51	3.18
	4c	1.45	0.44	0.74	1.13
	Mean ± SD	4.80 ± 2.12	0.94 ± 0.61	1.70 ± 2.13	1.80 ± 1.27
	Median (%)	100.00	18.79	18.79	49.32
	Quartile Range	0.00	19.06	37.70	37.70
A C 100	13a	7.27	1.55	0.75	5.63
	13b	4.82	0.75	0.74	6.45
	13c	3.26	1.00	1.50	0.75
	10a	6.45	7.27	3.18	0.75
	10b	4.82	1.55	0.75	0.75
	10c	7.27	13.87	1.00	1.00
	Mean ± SD	5.65 ± 1.61	4.33 ± 5.27	1.32 ± 0.96	2.56 ± 2.71
	Median (%)	100.00	31.53	15.51	19.34
	Quartile Range	0.00	91.33	32.37	63.68
A C 200	7a	4.82	0.75	0.75	0.75
	7b	7.27	7.27	1.00	0.75
	7c	7.27	0.76	0.75	1.50
	9a	4.00	0.75	0.75	1.00
	9b	4.82	1.00	1.00	1.00
	9c	9.74	2.37	0.75	11.27

	Mean ± SD	6.32 ± 2.16	2.15 ± 2.59	0.84 ± 0.13	2.71 ± 4.20
	Median (%)	100.00	19.80	14.69	20.74
	Quartile Range	0.00	8.71	8.46	9.45
U P 150	18a	5.63	4.82	0.75	0.75
	18b	4.00	0.75	0.75	0.75
	18c	6.45	0.75	0.75	0.75
	19a	5.63	0.74	0.74	0.75
	19b	4.82	0.75	0.75	0.75
	19c	3.18	0.75	4.00	0.75
	Mean ± SD	4.95 ± 0.25	1.43 ± 1.66	1.29 ± 1.33	0.75 ± 0.00
	Median (%)	100.00	17.20	14.47	14.47
	Quartile Range	0.00	10.43	5.61	5.45
U P 300	6a	4.00	0.75	0.75	0.75
	6b	5.63	1.50	1.50	0.75
	6c	2.37	0.75	0.75	0.75
	11a	4.82	0.75	3.18	0.75
	11b	4.00	0.75	1.50	1.50
	11c	2.37	0.75	4.51	0.75
	Mean ± SD	3.86 ± 1.31	0.88 ± 0.31	2.03 ± 1.50	0.88 ± 0.31
	Median (%)	100.00	22.74	34.66	25.26
	Quartile Range	0.00	12.94	39.41	16.13

Table 4.5.2: calculated insulin concentration results in µu/ml results for high calorie diet fed group.

		Baseline	1	2	3
	Rat code				
Control	1a	45.60	31.32	40.43	22.99
	1b	37.95	19.67	76.28	40.43
	1c	51.91	70.95	53.54	45.35
	2a	38.78	81.49	97.54	115.91
	2b	51.91	53.54	105.76	42.08
	2c	45.60	35.46	37.08	44.55
	Mean ± SD	45.29 ± 6.07	48.74 ± 24.14	68.44 ± 29.30	51.89 ± 32.43
	Median (%)	100	90.45	152.06	92.53
	Quartile Range	0	67.99	115.06	25.47
Metformin	3a	29.65	8.10	0.74	25.49
	3b	22.99	4.00	31.31	42.08
	3c	44.55	26.32	17.18	11.39
	4a	45.60	35.46	26.32	19.42
	4b	37.95	25.49	18.26	7.27
	4c	10.56	32.14	51.10	12.21
	Mean ± SD	31.89 ± 13.59	21.92 ± 12.90	24.15 ± 16.81	19.64 ± 12.76
	Median (%)	100	63.12	52.92	64.28
	Quartile Range	0	50.47	97.61	90.06
Mixture	5a	55.96	17.18	31.31	2.37
	5b	44.55	16.35	23.83	3.18
	5c	48.65	37.95	44.55	4.82
	6a	46.20	46.20	0.74	4.82
	6b	43.73	50.29	46.20	1.55
	6c	0.74	56.76	40.43	7.27
	Mean ± SD	39.97 ± 19.72	37.45 ± 17.15	31.18 ± 17.15	4.00 ± 2.07
	Median (%)	100	89	73.77	8.52
	Quartile Range	0	78.28	52.16	6.19
I T 50	7a	44.55	6.45	0.74	0.74
	7b	43.73	62.35	11.39	7.27
	7c	24.66	27.99	13.04	3.18
	8a	23.83	32.14	3.18	11.39
	8b	36.29	26.32	18.01	2.37
	8c	13.87	23.83	19.67	0.74

	Mean ± SD	31.16 ± 12.32	29.85 ± 18.23	11.01 ± 7.68	4.28 ± 4.22
	Median (%)	100	124.2	37.83	9.72
	Quartile Range	0	70.06	39.52	11.28
I T 100	9a	25.49	19.67	4.00	0.74
	9b	12.21	27.99	1.55	0.74
	9c	12.21	26.32	1.55	5.63
	10a	14.69	3.18	2.37	3.18
	10b	3.18	33.80	0.74	0.74
	10c	12.21	29.65	13.87	3.18
	Mean ± SD	13.33 ± 7.16	23.44 ± 10.95	4.01 ± 4.95	2.37 ± 2.00
	Median (%)	100	222.31	15.9	22.49
	Quartile Range	0	165.58	10.6	19.98
A C 100	11a	46.20	16.35	1.55	0.74
	11b	40.43	11.39	0.74	0.74
	11c	42.91	69.41	18.01	3.18
	12a	27.99	25.49	21.33	3.18
	12b	24.66	60.76	0.74	0.74
	12c	31.31	59.97	16.35	1.55
	Mean ± SD	35.58 ± 8.97	40.56 ± 25.62	9.79 ± 9.75	1.69 ± 1.20
	Median (%)	100	126.42	22.27	3.99
	Quartile Range	0	156.12	49.21	5.58
AC 200	13a	46.20	8.10	0.74	0.74
	13b	38.78	16.35	6.45	1.55
	13c	42.08	24.66	21.33	5.63
	14a	32.97	42.91	41.26	1.55
	14b	45.38	61.56	37.95	0.74
	14c	42.91	43.73	7.27	2.37
	Mean ± SD	41.39 ± 4.89	32.88 ± 19.98	19.17 ± 17.25	2.10 ± 1.84
	Median (%)	100	80.26	33.82	4.36
	Quartile Range	0	87.96	67	3.88
AC 600	15a	37.95	39.61	48.65	23.83
	15b	18.84	7.27	30.48	30.48
	15c	1.55	0.74	24.66	7.27
	16a	38.78	3.18	23.83	11.39
	16b	26.32	22.99	54.34	37.95
	16c	50.29	19.67	47.02	44.55
	Mean ± SD	28.96 ± 17.28	15.58 ± 14.78	38.16 ± 13.39	25.91 ± 14.67
	Median (%)	100	43.43	144.99	116.39

	Quartile Range	0	48.75	112.96	99.01
UP 150	17a	59.97	31.31	0.63	2.37
	17b	16.35	47.02	6.45	1.55
	17c	47.84	6.45	9.74	5.63
	18a	38.78	0.88	18.84	0.74
	18b	35.46	59.97	2.37	0.74
	18c	36.29	67.85	11.39	4.00
	Mean ± SD	39.12 ± 14.50	35.58 ± 27.70	8.24 ± 6.64	2.51 ± 1.96
	Median (%)	100	110.65	25.87	6.73
	Quartile Range	0	173	32.79	8.93
UP 300	19a	21.33	32.14	13.04	1.69
	19b	21.33	43.73	20.50	0.74
	19c	42.08	8.92	4.82	4.00
	20a	32.97	45.38	27.99	0.88
	20b	47.02	39.61	25.49	2.49
	20c	37.95	13.87	8.92	0.88
	Mean ± SD	33.78 ± 10.70	30.61 ± 15.65	16.79 ± 9.32	1.78 ± 1.28
	Median (%)	100	110.92	57.67	4.39
	Quartile Range	0	114.15	6138	5.24
U P 800	21a	21.33	7.27	30.48	13.87
	22b	13.04	12.21	22.99	40.43
	22c	55.96	44.55	0.74	55.96
	23a	34.64	34.64	0.74	50.29
	23b	11.39	51.10	23.83	8.10
	23c	0.74	5.63	5.63	3.18
	Mean ± SD	22.85 ± 19.77	25.90 ± 20.02	14.07 ± 13.20	28.64 ± 22.99
	Median (%)	100	96.83	159.61	122.59
	Quartile Range	0	369.07	207.06	238.98

Table 4.6.1 HOMA values in normal diet fed group

Week		Baseline	1	2	3
	(Rat code)				
Control	15a	2.28	2.80	3.15	5.97
	15b	2.03	2.28	5.65	4.43
	15c	1.26	3.30	1.96	4.08
	16a	1.57	5.17	3.64	3.74
	16b	1.97	2.99	4.44	4.10
	16c	1.68	3.74	6.71	5.55
	Mean ± SD	1.80 ± 0.37	3.38 ± 1.00	4.26 ± 1.72	4.65 ± 0.90
	Median (%)	100	187.1	228.33	150
	Quartile Range	0	139.6	121.84	106.89
Metformin	24a	1.73	0.21	0.21	0.21
	24b	0.33	0.29	0.28	0.58
	24c	0.98	0.25	1.26	1.26
	20a	1.33	0.29	0.28	0.50
	20b	1.54	0.21	1.00	1.35
	20c	0.78	0.66	1.12	0.56
	Mean ± SD	1.11 ± 0.52	0.61 ± 0.17	2.51 ± 0.48	0.77 ± 0.46
	Median (%)	100	23.36	75.36	79.62
	Quartile Range	0	0.078	107.39	90.95
Mixture	21a	2.03	0.21	0.21	0.21
	21b	1.38	0.18	0.91	0.24
	21c	0.89	0.24	0.21	0.29
	22a	1.78	1.59	0.37	0.24
	22b	1.18	0.37	0.13	0.32
	22c	1.12	0.21	0.32	0.21
	Mean ± SD	1.40 ± 0.43	0.47 ± 0.56	0.36 ± 0.28	0.25 ± 0.04
	Median (%)	100	22.68	22.14	17.97
	Quartile Range	0	18.51	17.05	13.47
I T 50	2a	0.98	0.23	0.38	1.25
	2b	0.74	0.60	0.50	0.29
	2c	1.57	0.34	0.18	0.68
	1a	1.12	0.26	0.21	0.34
	1b	1.77	0.18	0.18	0.19
	1c	1.37	0.54	0.16	1.45
	Mean ± SD	1.26 ± 0.38	0.36 ± 0.17	0.27 ± 0.14	0.70 ± 0.53
	Median (%)	100	23.54	15	40
	Quartile Range	0	18.37	27.5	75.62
I T 100	3a	1.77	0.35	0.26	1.11
	3b	1.80	0.29	0.21	0.26
	4a	0.98	0.16	0.21	0.24
	4b	1.58	0.16	1.93	1.11

	4c	0.30	0.09	0.21	0.35
	Mean ± SD	1.29 ± 0.78	0.21 ± 0.09	0.56 ± 0.85	0.62 ± 0.67
	Median (%)	100	16.11	21.47	62.77
	Quartile Range	0	3.75	53.23	46.3
A C 100	13a	2.54	0.33	0.16	1.97
	13b	1.51	0.26	0.16	1.58
	13c	1.02	0.21	0.32	0.34
	10a	1.80	1.78	1.11	0.21
	10b	1.51	0.54	0.16	0.34
	10c	2.29	4.36	0.21	0.25
	Mean ± SD	1.78 ± 0.56	1.25 ± 1.63	0.35 ± 0.38	0.78 ± 0.78
	Median (%)	100	28.19	10.34	27.94
	Quartile Range	0	81.28	21.58	65.3
A C 200	7a	1.01	0.16	0.21	0.34
	7b	2.29	2.80	0.18	0.34
	7c	2.54	0.26	0.16	0.63
	9a	1.12	0.21	0.13	0.53
	9b	1.18	0.46	0.21	0.49
	9c	3.06	0.58	0.21	5.12
	Mean ± SD	1.87 ± 0.88	0.74 ± 1.02	0.18 ± 0.03	1.29 ± 1.90
	Median (%)	100	18.85	9.7	37.74
	Quartile Range	0	23.04	10.97	22.19
U P 150	18a	1.77	1.35	0.24	0.26
	18b	1.26	0.18	0.21	0.21
	18c	1.58	0.18	0.18	0.26
	19a	1.18	0.18	0.21	0.24
	19b	1.18	0.16	0.21	0.29
	19c	1.11	0.16	1.40	0.24
	Mean ± SD	1.35 ± 0.27	0.37 ± 0.48	0.41 ± 0.49	0.25 ± 0.03
	Median (%)	100	14.39	17.14	18.35
	Quartile Range	0	2	4.5	4.61
U P 300	6a	0.98	0.16	0.18	0.29
	6b	0.98	0.37	0.53	0.21
	6c	0.66	0.18	0.24	0.18
	11a	1.01	0.18	0.89	0.18
	11b	0.70	0.26	0.37	0.42
	11c	0.66	0.18	1.73	0.34
	Mean ± SD	0.83 ± 0.17	0.22 ± 0.08	0.66 ± 0.59	0.27 ± 0.10
	Median (%)	100	27.77	53.00	28.65
	QuartileRange	0	19.14	52.42	30.23

Table 4.6.2 HOMA values in high calorie diet fed group

		Baseline	1	2	3
	Rat code				
Control	1a	17.05	12.77	13.74	9.38
	1b	16.77	7.35	72.60	16.49
	1c	22.94	28.94	30.93	21.58
	2a	15.82	38.78	39.79	43.34
	2b	21.17	20.02	43.14	20.03
	2c	15.50	14.47	27.73	12.12
	Mean ± SD	18.21 ± 3.09	20.39 ± 11.61	37.99 ± 19.85	20.49 ± 12.11
	Median	100	93.93	191.31	94.33
	Quartile Range	0	51.23	116.68	20.17
Metformin	3a	17.13	3.03	0.33	8.66
	3b	9.38	1.09	18.09	10.01
	3c	18.17	8.05	9.34	4.26
	4a	21.70	12.05	12.53	6.60
	4b	11.61	11.26	5.59	2.97
	4c	4.31	14.20	29.53	3.32
	Mean ± SD	13.72 ± 6.43	8.28 ± 5.25	12.57 ± 10.27	5.97 ± 2.93
	Median	100	49.93	54.57	40.5
	Quartile Range	0	79.35	144.79	51.52
Mixture	5a	11.73	6.60	13.13	0.74
	5b	9.34	5.14	7.49	0.89
	5c	10.20	13.26	18.68	1.35
	6a	8.07	19.37	0.31	1.35
	6b	10.69	17.57	24.21	0.54
	6c	0.21	23.80	14.13	3.05
	Mean ± SD	8.37 ± 4.19	14.29 ± 7.36	12.99 ±8.37	1.32 ± 0.91
	Median	100	147.14	147.53	11.36
	Quartile Range	0	183.71	146.15	10.33
I T 50	7a	10.90	2.71	0.36	0.39
	7b	15.28	15.25	2.79	2.29
	7c	7.75	6.84	7.29	0.67
	8a	4.99	11.23	1.89	3.18
	8b	11.41	7.36	9.44	0.41
	8c	3.88	7.49	6.18	0.18
	Mean ± SD	9.04 ± 4.31	8.48 ± 4.28	4.66 ± 3.51	1.19 ± 1.24
	Median	100	94.03	60.28	6.66
	Quartile Range	0	128.83	75.79	11.34
I T 100	9a	4.45	5.50	1.12	0.16
	9b	3.41	6.84	0.65	0.47
	9c	2.56	8.28	0.87	1.57
	10a	4.62	0.89	1.24	0.78

	10b	0.78	11.81	0.21	0.16
	10c	4.27	10.36	6.78	2.00
	Mean ± SD	3.35 ± 1.48	7.28 ± 3.88	1.81 ± 2.46	0.86 ± 0.77
	Median	100	221.62	26.76	18.42
	Quartile Range	0	199.78	8.84	33.233
A C 100	11a	11.30	5.71	0.60	0.18
	11b	9.89	4.38	0.34	0.21
	11c	13.49	21.82	8.81	0.78
	12a	6.84	9.80	6.71	1.00
	12b	5.17	27.60	0.49	0.21
	12c	9.85	23.05	15.42	0.38
	Mean ± SD	9.42 ± 3.01	15.39 ± 9.95	5.39 ± 6.11	0.46 ± 0.35
	Median	100	152.44	37.41	3.94
	Quartile Range	0	183.51	92.71	3.67
AC 200	13a	11.30	2.83	0.26	0.29
	13b	8.13	5.14	1.80	0.43
	13c	10.29	10.34	5.96	4.53
	14a	8.06	14.99	10.09	0.92
	14b	11.10	27.96	7.96	0.42
	14c	10.49	18.33	2.54	1.24
	Mean ± SD	9.90 ± 1.44	13.27 ± 9.25	4.77± 3.85	1.30 ± 1.62
	Median	100	137.58	41.07	8.4
	Quartile Range	0	122.65	49.5	8.09
AC 600	15a	11.93	12.45	28.90	7.49
	15b	6.58	2.29	13.84	10.65
	15c	0.54	0.29	12.06	2.80
	16a	17.61	1.11	14.98	3.58
	16b	14.71	8.84	28.48	9.28
	16c	21.08	6.18	23.00	12.45
	Mean ± SD	12.08 ± 7.51	5.19 ± 4.82	20.21 ± 7.56	7.71 ± 3.87
	Median	100	43.64	201.94	62.93
	Quartile Range	0	30.72	133.07	102.72
UP 150	17a	18.86	8.75	0.24	0.41
	17b	4.57	36.14	2.03	0.76
	17c	11.70	2.03	6.13	1.38
	18a	9.48	0.18	7.24	0.23
	18b	8.67	23.05	1.41	0.21
	18c	10.14	21.34	5.17	1.40
	Mean ± SD	10.57 ± 4.71	15.25 ± 13.99	3.70 ± 2.85	0.73 ± 0.55
	Median	100	128.37	47.7	7.12
	Quartile Range	0	248.37	36.14	11.38
UP 300	19a	5.96	12.35	8.66	0.59
	19b	5.96	15.28	5.73	0.23

	19c	10.29	2.18	1.35	0.98
	20a	8.06	9.51	10.76	0.28
	20b	13.14	11.07	8.91	0.61
	20c	10.61	4.36	4.36	0.34
	Mean ± SD	9.00 ± 2.85	9.13 ± 4.96	6.63 ± 3.47	0.50 ± 0.28
	Median	100	101.1	81.94	4.28
	Quartile Range	0	166.08	92.25	6.08
U P 800	21a	6.71	2.54	17.04	3.88
	22b	5.01	6.40	13.66	16.95
	22c	37.15	21.79	0.42	19.55
	23a	16.94	14.52	0.44	17.57
	23b	5.17	19.64	13.32	1.13
	23c	0.34	2.17	3.94	0.89
	Mean ± SD	11.89 ± 13.54	11.18 ± 8.65	8.13 ± 7.39	9.99 ± 8.90
	Median	100	106.72	255.76	80.744
	Quartile Range	0	320.99	269.91	211.2

Table 4.7.1 calculated free fatty acid concentration results in mmol/l for normal diet fed group.

Week	Week	Baseline	1	2	3
	(Rat code)				
Control	15a	1.96	1.44	1.70	2.02
	15b	0.87	1.70	1.84	1.79
	15c	0.76	1.50	1.90	0.81
	16a	1.39	2.10	1.80	1.39
	16b	1.52	2.10	1.30	0.82
	16c	0.87	1.50	1.25	0.79
	Mean ± SD	1.23 ± 0.47	1.72 ± 0.30	1.63 ± 0.28	1.27 ± 0.55
	Median (%)	100	161.75	136.59	101.53
	Quartile Range	0	57.24	124.76	15.77
Metformin	24a	1.15	0.32	0.62	1.12
	24b	0.42	0.37	0.98	1.12
	24c	1.13	0.29	0.71	0.86
	20a	0.44	0.14	0.35	0.92
	20b	1.40	0.14	0.98	0.69
	20c	1.00	0.23	0.72	0.74
	Mean ± SD	0.92 ± 0.40	0.25 ± 0.10	0.73 ± 0.24	0.91 ± 0.18
	Median (%)	100	26.75	71	86.77
	Quartile Range	0	8.82	16.71	135.1
Mixture	21a	0.27	0.59	1.49	0.46
	21b	0.46	1.84	0.78	0.96
	21c	0.83	1.28	1.59	0.89
	22a	0.69	0.93	1.05	1.55
	22b	1.08	0.24	1.60	1.3
	22c	0.96	0.31	0.85	1.8
	Mean ± SD	0.72 ± 0.31	0.87 ± .62	1.23 ± 0.38	1.16 ± 0.49
	Median (%)	100	144.5	160.87	178.94
	Quartile Range	100	186.23	43.42	88.33
I T 50	2a	1.52	1.03	0.86	0.91
	2b	1.17	0.93	0.94	0.88
	2c	0.47	2.40	1.77	1.58
	1a	1.15	2.18	0.88	0.91

	1b	0.42	1.09	0.94	0.85
	1c	1.28	1.50	1.20	1.5
	Mean ± SD	1.00 ± 0.45	1.52 ± 0.63	1.10 ± 0.35	1.11 ± 0.34
	Median (%)	100	153.38	87.05	98.16
	Quartile Range	0	180.04	147.28	127.17
I T 100	3a	1.13	1.13	0.89	0.92
	3b	1.20	0.93	1.15	1.19
	4a	1.33	1.40	1.96	1.84
	4b	0.85	1.96	1.17	0.96
	4c	1.00	1.00	1.43	1.45
	Mean ± SD	1.10 ± 0.18	1.28 ± 0.42	1.32 ± 0.41	1.27 ± 0.38
	Median (%)	100	100	137.65	112.94
	Quartile Range	0	5.26	47.17	39.18
A C 100	13a	1.20	2.03	1.55	1.61
	13b	1.18	0.96	1.35	1.25
	13c	1.01	1.01	0.91	1.1
	10a	0.96	1.57	0.85	0.95
	10b	1.15	1.35	1.37	1.2
	10c	1.79	1.13	1.59	1.64
	Mean ± SD	1.22 ± 0.30	1.34 ± 0.41	1.27 ± 0.32	1.29 ± 0.28
	Median (%)	100	108.7	102.25	105.14
	Quartile Range	0	82.19	30.3	9.95
A C 200	7a	1.57	2.00	1.31	1.21
	7b	1.01	1.03	1.04	0.95
	7c	0.91	1.12	1.30	1.19
	9a	0.81	0.93	1.91	1.55
	9b	0.95	0.76	1.01	0.85
	9c	2.00	1.32	1.40	1.25
	Mean ± SD	1.21 ± 0.43	1.19 ± 0.40	1.33 ± 0.30	1.17 ± 0.25
	Median (%)	100	108.4	104.64	91.77
	Quartile Range	0	43.08	59.42	53.7
U P 150	18a	0.47	0.90	0.91	1.06
	18b	0.91	1.01	1.55	1.27
	18c	0.36	1.17	1.65	1.49
	19a	0.78	0.69	1.45	1.37

	19b	1.37	1.40	1.60	1.44
	19c	0.64	0.91	0.29	0.32
	Mean ± SD	0.76 ± 0.36	1.01 ± 0.25	1.24 ± 0.54	1.15 ± 0.44
	Median (%)	100	126.59	178.11	157
	Quartile Range	0	89.3	76.83	120.42
U P 300	6a	0.96	0.28	0.86	0.31
	6b	0.63	0.64	1.16	1.33
	6c	0.26	0.56	1.06	0.42
	11a	0.53	0.51	1.19	0.31
	11b	0.56	0.29	1.34	0.86
	11c	0.71	0.31	0.8	0.51
	Mean ± SD	0.61 ± 0.23	0.43 ± 0.16	1.07 ± 0.21	0.62 ± 0.40
	Median (%)	100	74	204.32	112.7
	Quartile Range	0	57.93	126.61	103.05

Table 4.7.2 calculated free fatty acid concentration results in mmol/l for special diet fed group.

	Rat code	Baseline	1	2	3
Control	1a	1.39	1.30	1.87	1.12
	1b	0.79	1.39	1.42	1.52
	1c	0.85	1.15	1.50	2.08
	2a	1.84	1.08	1.90	1.35
	2b	0.83	2.01	2.50	2.31
	2c	1.93	1.74	2.40	1.64
	Mean ± SD	1.27 ± 0.52	1.45 ± 0.36	1.93 ± 0.45	1.67 ± 0.45
	Median	100	114.41	155.5	138.68
	Quartile Range	0	85.79	55.4	164.13
Metformin	3a	1.07	1.30	1.40	1.35
	3b	1.03	2.09	1.60	1.42
	3c	1.62	1.67	1.40	1.31
	4a	1.20	1.47	1.57	1.43
	4b	1.70	0.91	1.12	1.05

	4c	1.84	2.07	1.50	1.32
	Mean ± SD	1.41 ± 0.35	1.59 ± 0.46	1.43 ± 0.17	1.31 ± 0.14
	Median	100	117	108.63	100.02
	Quartile Range	0	19.41	49.32	54.43
Mixture	5a	0.73	0.86	1.18	1.14
	5b	0.93	1.33	1.12	0.43
	5c	1.03	1.40	0.91	1.39
	6a	0.78	1.20	1.33	1.89
	6b	0.74	1.12	0.95	1.17
	6c	1.08	1.12	0.56	1.07
	Mean ± SD	0.88 ± 0.15	1.17 ± 0.19	1.01 ± 0.27	1.18 ± 0.47
	Median	100	139.47	124.4	145.56
	Quartile Range	0	33.54	73.29	59.03
I T 50	7a	0.82	0.56	1.28	0.88
	7b	0.81	1.13	1.37	1.06
	7c	1.04	0.73	0.78	1.6
	8a	0.56	0.80	1.00	0.56
	8b	0.82	0.85	0.78	1.3
	8c	0.71	0.88	0.96	1.76
	Mean ± SD	0.79 ± 0.16	0.825 ± 0.19	1.03 ± 0.25	1.19 ± 0.45
	Median	100	113.8	145.65	142.36
	Quartile Range	0	69.31	74.01	51.22
I T 100	9a	1.12	1.23	0.90	1.89
	9b	1.92	1.31	1.50	0.76
	9c	1.30	0.99	0.63	1.55
	10a	1.23	1.89	1.25	0.81
	10b	1.49	0.86	0.81	1.81
	10c	1.45	1.40	1.40	0.81
	Mean ± SD	1.42 ± 0.28	1.28 ± 0.36	1.08 ± 0.35	1.27 ± 0.54
	Median	100	86.35	79.24	92.54
	Quartile Range	0	41.59	42.19	65.61
A C 100	11a	0.54	2.29	1.05	1.55
	11b	0.78	0.98	0.78	0.86
	11c	0.71	1.86	1.20	0.92
	12a	0.91	0.86	0.79	0.81

	12b	0.49	1.01	0.88	1.11
	12c	0.61	0.78	0.85	1.2
	Mean ± SD	0.67 ± 0.16	1.30 ± 0.62	0.93 ± 0.17	1.08 ± 0.98
	Median	100	167	154.18	163.15
	Quartile Range	0	136.33	79.59	116.27
AC 200	13a	0.73	0.98	0.76	0.98
	13b	0.85	0.56	0.79	0.75
	13c	1.04	0.66	1.05	0.86
	14a	0.93	0.81	0.87	1.05
	14b	1.28	0.91	0.95	0.56
	14c	1.04	0.99	0.85	1.15
	Mean ± SD	0.98 ± 0.19	0.82 ± 0.18	0.88 ± 0.11	0.89 ± 0.21
	Median	100	79.09	93.25	99.41
	Quartile Range	0	29.31	19.23	30.21
AC 600	15a	1.20	1.89	1.70	1.27
	15b	1.32	1.64	1.42	1.55
	15c	1.28	2.31	2.40	1.86
	16a	2.30	2.03	1.60	1.41
	16b	1.20	2.40	2.33	1.34
	16c	2.80	0.81	1.01	1.21
	Mean ± SD	1.68 ± 0.69	1.85 ± 0.58	1.58 ± 0.86	1.44 ± 0.24
	Median	100	140.87	124.62	108.75
	Quartile Range	0	92.21	117.93	56.12
UP 150	17a	1.00	0.95	0.78	0.44
	17b	0.68	0.85	1.40	0.63
	17c	1.32	1.50	0.88	1.1
	18a	0.54	1.40	1.79	1.23
	18b	0.71	1.12	1.27	1.33
	18c	1.08	1.10	1.60	1.74
	Mean ± SD	0.89 ± 0.29	1.15 ± 0.25	1.29 ± 0.40	1.08 ± 0.47
	Median	100	119.32	163.51	126.88
	Quartile Range	0	55.9	127.88	103.99
UP 300	19a	0.81	1.60	0.54	1.14
	19b	0.64	1.60	1.01	1.4
	19c	0.88	1.20	0.66	1.01

	20a	0.82	1.63	0.86	1.12
	20b	1.03	1.04	1.10	1.08
	20c	0.79	1.10	0.88	0.41
	Mean ± SD	0.83 ± 0.13	1.36 ± 0.28	0.84 ± 0.21	1.03 ± 0.33
	Median	100	168.39	105.84	125.68
	Quartile Range	0	62.42	36.39	35.89
U P 800	21a	0.59	1.23	1.48	1.41
	22b	1.50	1.81	1.50	1.35
	22c	1.44	2.16	1.44	1.52
	23a	2.20	2.08	1.66	1.48
	23b	1.22	1.13	1.13	1.15
	23c	1.04	1.14	1.20	0.98
	Mean ± SD	1.33 ± 0.54	1.59 ± 0.48	1.40 ± 0.20	1.32 ± 0.21
	Median	100	115.14	100	94.27
	Quartile Range	0	55.55	22.76	15.56