EFFECT OF DIETARY *TERMINALIA SERICEA* AQUEOUS LEAF EXTRACTS ON HIGH-FRUCTOSE DIET FED GROWING WISTAR RATS

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DECLARATION

I, **Busisani Wiseman Lembede**, declare that this dissertation is my own work. It is being submitted for the degree of Master of Science in Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

……

Busisani Wiseman Lembede

Signed on the……..day of…….2014

DEDICATION

To my late grandmother

Thembeni Alizina Lembede

1928 - 2013

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ABSTRACT

Sedentary lifestyles and poor dietary choices are the major cause of the global increase in the prevalence of obesity and metabolic dysfunction in children. The high cost and limited access to conventional drugs by poor communities make them depend on ethnomedicines. *Terminalia sericea (T. sericea)* contains phytochemicals that give its extracts hypolipidaemic and hypoglycaemic properties hence its use in ethnomedicine to treat diabetes mellitus. Using weanling Wistar rat pups fed a high fructose diet to model growing children exposed to high-sugar diets, this study sought to evaluate the effects of aqueous *T. sericea* leaf extracts on their growth performance, glucose homeostasis, visceral morphometry and their general health profile. Forty 21-day old male Wistar pups were randomly allocated to five treatment regimens. Each group had *ad libitum* access to a commercially supplied rat chow. Group 1 pups were given plain drinking water and plain gelatine cubes, group 2: 12% fructose solution and plain gelatine cubes, group 3: 12% fructose solution and gelatine cubes containing fenofibrate at a dosage of 100 $mg.kg^{-1}$ per day, group 4: 12% fructose solution and gelatine cubes with a low dose (100 $mg.kg^{-1}$ per day) of the *T. sericea* extract and group 5: 12% fructose solution and gelatine cubes with a high dose (400 $mg.kg^{-1}$ per day) of the *T. sericea* extract. The pups were maintained on the regimens for 12 weeks after which they under went an oral glucose tolerance test. Fasting blood metabolite content was then determined after which the rats were killed and tissues collected for visceral morphometrical, linear growth and surrogate markers' of health determinations.

T. sericea extracts had no negative effect on growth performance (body mass and indexes of long bone growth) but rats given fenofibrate had lighter empty carcasses and shorter tibiae.

The administration of *T. sericea* extracts neither improved glucose homeostasis nor caused derangement of glucose handling by rats given a high fructose diet following an oral glucose challenge. However, the administration of fenofibrate to rats given a high fructose diet resulted in decreased glucose handling following an oral glucose challenge. With the exception of the administration of fenofibrate which resulted in a significantly high ($P < 0.05$) fasting blood glucose concentration, treatment regimens had no effect on fasting blood glucose, triglyceride and cholesterol concentrations. Rats given fructose with either plain gelatine cubes or low *T. sericea* dose had significantly higher ($P < 0.05$) liver lipid content compared with the control treatment. Administration of *T. sericea* extracts to rats given a high fructose diet had no effect on the GIT, other abdominal viscera and markers of general health. The administration of fenofibrate to rats given a high fructose diet caused increased relative mass of GIT organs (stomach, small intestine and caecum), increased absolute mass of other viscera (liver and kidney); increased serum phosphorus and alkaline phosphatase concentration.

Results from the study revealed that administration of a high dose of aqueous *T. sericea* leaf extracts has potent phytochemicals properties that has helped to prevent high fructose diet-induced deposition of fat in the in the liver (non-alcoholic fatty liver disease), without compromising growth, visceral morphometry and general health of growing Wistar rats.

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LIST OF ABBREVIATIONS

α:	Alpha
β:	Beta
ALP:	Alkaline phosphatase
ALT:	Alanine aminotransferase
ANOVA:	Analysis of variance
ATP:	Adenosine triphosphate
BM:	Body mass
BUN:	Blood urea nitrogen
COX:	Cyclooxygenase
DM:	Dry matter
FF:	Fenofibrate in gelatine cube
FFAs:	Free fatty acids
FS:	Fructose solution
GIT:	Gastro intestinal tract
HD:	High dose aqueous *Terminalia sericea* leaf extract in gelatine cube
HDL:	High density lipoprotein

HOMA-IR:	Homeostatic model of insulin resistance
IDF:	International Diabetes Federation guidelines
LD:	Low dose aqueous *Terminalia sericea* leaf extract in gelatine cube
LDL:	Low density lipoprotein
LI:	Large intestines
OGTT:	Oral glucose tolerance test
PC:	Plain gelatine cube
PW:	Plain water
PPAR:	Peroxisome proliferator activated receptor
SI:	Small intestine
TLr:	Relative tibia length
TBIL:	Total bilirubin
TNF:	Tumour necrosis factor
VLDL:	Very low density lipoprotein
w/v:	weight/volume
WHO:	World Health Organisation

CHAPTER 1: INTRODUCTION

1.0 Introduction

Urbanisation has led to an increase in the proportion of children and adolescents living sedentary lifestyles and consuming artificially sweetened foods, rich in fructose (Rutledge and Adeli, 2007; Weiss *et al.,* 2004). The consumption of diets high in fructose has been shown to cause obesity and metabolic syndrome in South African adolescents (Steyn and Temple, 2012). Metabolic syndrome is a manifestation of various medical conditions (Weiss *et al.,* 2004) that include abdominal obesity, atherogenic dyslipidaemia, hypoalbuminaemia, raised blood pressure, insulin resistance, glucose intolerance and hypercholesterolaemia (Alberti *et al.*, 2006; Huang *et al.*, 2004). It has been suggested that obesity is the main driver of increased childhood metabolic syndrome, in developing countries (Misra and Khurana, 2008; Kelishadi, 2007). In South Africa the prevalence of childhood obesity is estimated to be 7.8% and it is expected to increase to 22.8% by 2020 (Toriola *et al.,* 2012). The expected increase in childhood obesity will cause an increase in the prevalence of metabolic syndrome as a consequence (Mollentze, 2006).

Pharmaceutical drugs such as metformin and fenofibrates are used to manage metabolic syndrome (Kraja *et al.,* 2010; Kirpichnikov *et al.,* 2002). Despite the existence of pharmaceutical drugs, about 80% of the population in communities in the developing countries rely on medicinal plants to treat ailments such as metabolic dysfunction (Fyhrquist *et al.,* 2002). This is due to the high cost, limited access and availability pharmaceutical drugs to third world country communities. The tree *Terminalia sericea* (*T. sericea*) is widely distributed in subtropical Africa and is used by traditional healers to treat wounds, stomach problems, hypertension and diabetes

mellitus (Mochizuki and Hasegawa 2007; Fyhrquist *et al.,* 2002). Its extracts contain biologically and pharmacologically active phytochemicals including tannins, saponins and flavonoids (Nkobole *et al.,* 2011; Mochizuki and Hasegawa 2006). Tannins, saponins and flavonoids have been demonstrated to have antioxidant, antidiabetic, antimicrobial and cardioprotective properties (Sandhar *et al.,* 2011; Subramaniam *et al.,* 2011; Dwivedi 2007; Eckel *et al.,* 2005). *T. sericea* is used in ethnomedicine to treat diabetes mellitus (Fyhrquist *et al.,* 2002), however only *in-vitro* studies have been done to evaluate its hypoglycaemic, antidiabetic and hypolipidaemic properties (Nkobole *et al.,* 2011; Mochizuki and Hasegawa, 2006).

1.1 Justification of study

Majority of studies evaluating the antidiabetic and antiobesity potential of medicinal plants used streptozotocin-induced diabetic rat models to evaluate the efficacy of the plants (Din *et al.,* 2011; Njomen *et al.,* 2009; Rao and Nammi, 2006). Streptozotocin-induced diabetic rat models do not efficiently mimic the metabolic syndrome in humans caused by the consumption of diets high in refined sugars (Huang *et al.,* 2004) hence it is preferable to use high fructose diet-induced metabolic syndrome rat models (Huang *et al.,* 2004). In addition most studies have used adult rats and have focused on trying to find curative traditional plants (Njomen *et al.,* 2009; Rao and Nammi, 2006). However, the prevalence of metabolic syndrome and obesity has been identified to be increasing in children and adolescents (Kelishadi, 2007) hence the need to use growing animals (i.e growing rats) modelling the development of diet-induced obesity as well as

metabolic dysfunction in children and adolescents (Kohen-Avramoglu *et al.,* 2003) with aim of exploring preventative interventions.

Although there are many conventional pharmaceutical drugs that can be used in treating metabolic syndrome and obesity, their high cost and poor accessibility especially for third world country communities has led to the resurgence of research focusing on indigenous trees and herbs as potential treatments for metabolic syndrome (Ayyanar *et al.,* 2008). *T. sericea* is an indigenous tree with multiple ethnomedicinal uses (Deutschlander *et al.,* 2009; Mochizuki and Hasegawa 2007) including the treatment of diabetes mellitus (Deutschlander *et al.,* 2009; Fyhrquist *et al.,* 2002). *In vitro* studies have reported that *T. sericea* stem bark and root to have anti-diabetic and anti-obesity properties (Nkobole *et al.,* 2011; Mochizuki and Hasegawa 2006). *T. sericea* aqueous leaf extracts contain saponins, tannins and flavonoids (Masoko *et al.,* 2005; Bessong *et al.,* 2004; Fyhrquist *et al.,* 2002). Saponins, tannins and flavonoids are known to have hypoglycaemic, anti-diabetic and lipolytic properties thus *T. sericea* could have the potential to prevent the onset of fructose-induced metabolic dysfunction and obesity. There is little or no research that has been done regarding *T. sericea* leaves as a potential preventative medication for high fructose diet-induced metabolic dysfunction and obesity in growing rats modelling human children and adolescents.

The first point of contact for orally administered substances is the gastrointestinal tract (GIT) (Rubino *et al.,* 2010). Therefore, the first place where the plant extracts exert their effects would be in the GIT. The ontogenic development of the GIT has three-stages: the prenatal, neonatal and

post-weaning stage (Zabielski *et al.,* 2008). Prenatally there is little stimulation of the GIT lumen (Zabielski *et al.,* 2008). Neonatal stimulation is by milk and the post-weaning stage is when the switch from milk to solid feed occurs (Zabielski *et al.,* 2008). The post-weaning stage is a critical stage in the development of the GIT. The introduction of solid feeds too early (to the not yet adapted GIT of infants) increases the risk of developing GIT post-weaning disorders (Zabielski *et al.,* 2008). The GIT is vital in the digestion and absorption of nutrients; altering nutrient absorption can affect overall growth and development of the growing animal (Pérez *et al.,* 2007).

Compounds found in plant extracts used in ethnomedicines can exert negative or positive effects on the nutrient absorptive capacity of the GIT (González-Alvarado *et al.,* 2007; Kochhar *et al.,* 2007). *T. sericea* leaves are rich in tannins (Nkobole *et al.,* 2011). Studies have suggested that tannins may have detrimental effects on the mucosal layer of the GIT, resulting in reduced nutrient absorption and reduced growth in animals (Chung *et al.,* 1998). However, Mochizuki and Hasegawa (2007) showed that *T. sericea* sericosides have protective properties within the GIT lumen. Thus, administration of *T. sericea* orally can potentially have positive or negative effects on the development of the GIT, as well as nutrient absorption. Furthermore, medicinal plants used in communities are usually folk referrals and are believed to be harmless to the body (Erasto *et al.,* 2005). However, medicinal plants have the potential to cause adverse side effects such as cytotoxicity (Bussmann *et al.,* 2011; Kane *et al.,* 1995). Hence, when researching medicinal properties of plants there is a need to also screen them for toxicity.

1.2 Aim of study

The broad objective of the current study was to determine the effects of orally administered aqueous *T. sericea* leaf extracts in high-fructose diet-fed growing Wistar rats. Specifically the study sought to determine the effects of orally administered aqueous *T. sericea* leaf extracts in growing Wistar rats on:

a. growth performance (body mass and indexes of long bone growth).
b. glucose tolerance.
c. serum concentrations of metabolic substrates (glucose, triglycerides, free fatty acids and cholesterol).
d. serum insulin concentration and HOMA IR index.
e. liver glycogen and lipid storage.
f. the general health profile by determining serum activities of alanine aminotransferase (ALT) and alkaline phosphatase (ALP) and other markers (serum creatinine, urea, total bilirubin, globulin, calcium, phosphorus, total protein, albumin, cholesterol, and amylase) of general health.
g. GIT and visceral organ macro- and micro-morphometry.
h. To determine the phytochemical composition and toxicity of aqueous *T. sericea* leaf extracts, using qualitative phytochemical screening and the brine shrimp toxicity assay.

1.3 Hypothesis

The null and alternate hypotheses of the study were:

i. H_0: Orally administered aqueous *T. sericea* leaf extracts do not improve the growth performance, post-weaning GIT development and have no effect on markers of metabolic dysfunction and the general health profile of growing male Wistar rats given a high fructose diet.

ii. H_1: Orally administered aqueous *T. sericea* leaf extracts improve growth performance, post-weaning GIT development and affect markers of metabolic dysfunction and the general health profile of growing male Wistar rats given a high fructose diet.

CHAPTER 2: LITERATURE REVIEW

2.0 Introduction:

Metabolic syndrome refers to the manifestation of a combination of risk factors (visceral obesity, insulin resistance, hyperglycaemia, hypertriglyceridaemia, increased low density lipoprotein, decreased high density lipoprotein and hypertension) that increase the chance of developing type II diabetes mellitus and cardiovascular diseases (Beilby, 2004). According to the International Diabetes Federation guidelines (IDF) and World Health Organisation (WHO) guidelines, metabolic syndrome in children and adolescents is diagnosed when patients present with central obesity and any other 2 of hyperglycemia, hypertriglyceridaemia, hypertension and decreased high density lipoprotein (Friend *et al.,* 2013). In developing countries the number of children and adolescents that are obese has been increasing rapidly thus more children and adolescents are at risk of developing metabolic dysfunction (Al-Isa, 2013). Metabolic dysfunction is associated with altered insulin secretion, insulin resistance and type II diabetes mellitus (Al-Isa, 2013).

2.1 Consequences of metabolic syndrome

Insulin resistance, an inability of target cells to respond appropriately to insulin (Mather *et al*., 2007), and hyperglycaemia are the main mediators of the consequences of metabolic syndrome. It (insulin resistance) results in hyperglycaemia which leads to dyslipidaemia (Gall *et al.,* 2010). The latter is characterised by higher than normal plasma concentrations of triglycerides, cholesterol and low density lipoproteins (Li *et al.,* 2011) which is driven by the greater esterification of free fatty acids (FFAs) compared to their oxidation (Thirunavukkarasu *et al.*,

2004). Increased esterification of FFAs promotes the formation and secretion of triglycerides, cholesterol and very low density lipoprotein (VLDL) by the liver (Rutledge and Adeli, 2007; Thirunavukkarasu *et al.,* 2004). Very low density lipoprotein (VLDL) is known to be inefficient at clearing plasma cholesterol (Thirunavukkarasu *et al.,* 2004), it transports the cholesterol from the liver to skeletal and cardiac muscle as well as adipose tissue (Choi and Ginsberg, 2011). The increase in triglyceride, cholesterol and non-high density lipoproteins increases inflammatory cytokines such as tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) (Wajchenberg *et al.,* 2009). An increase in inflammatory cytokines combined with dyslipidaemia, hyperglycaemia and insulin resistance can lead to the onset type II diabetes mellitus and its complications (LaRosa *et al.,* 2013; Matsudaa and Shimomurab, 2013).

2.1.1 Diabetes mellitus

Worldwide, diabetes mellitus is ranked as the third most common chronic disease in children (Pettitt *et al.,* 2013). Diabetes mellitus is a metabolic disorder charecterised by increased blood glucose concentration (Pettitt *et al.,* 2013). The two most common subtypes of diabetes mellitus are Type I and II diabetes mellitus. Type I diabetes occurs due to autoimmune destruction of β-cells of the pancreas (Herold *et al.,* 2002) while type 2 occurs due to altered insulin secretion and resistance by body tissue (Neergheen-Bhujun *et al.,* 2013). In 2011, an estimated 366 million people had diabetes mellitus worldwide. This figure is expected to increase to 552 million by 2030 (Al-Sarihin *et al.,* 2013). In sub-Suharan Africa, about 12.1 million people had diabetes mellitus

(Chen *et al.*, 2011a), with an estimated increase to 23.9 million by 2030 (Chen *et al.*, 2011a; Mbanya *et al.*, 2010).

2.1.2 Insulin secretion and complications of type II diabetes mellitus

Insulin is a peptide hormone produced by the β-cells of the endocrine pancreas (Inzucchi and Sherwin, 2011). It is a major anabolic hormone that regulates metabolism (Dentin *et al.*, 2007). It promotes the uptake and storage of glucose and lipids in the liver, muscle and adipose tissue (Qu *et al.*, 2011). The secretion of insulin is stimulated by increase blood glucose concentration (Qu *et al.*, 2011). The mechanism of insulin secretion is as summarised in Figure 1.1 below.

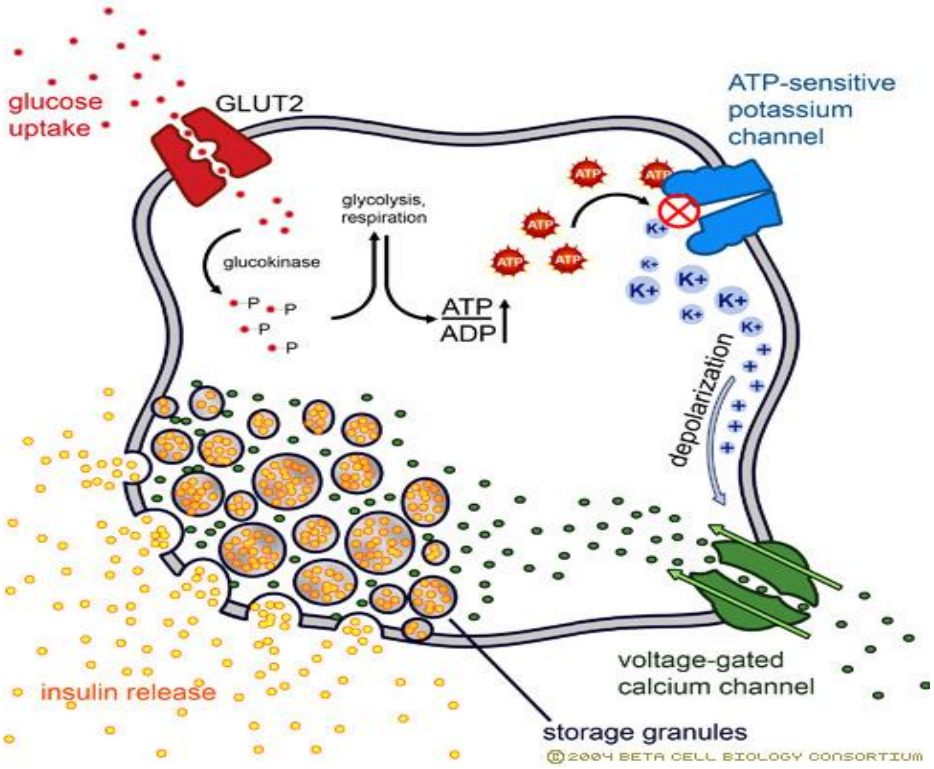

Figure1.1: A Schematic representation of the insulin secretion mechanism

Glucose enters the β-cell from the blood by facilitation of GLUT 2 transporters (Scheepers *et al.,* 2004). The increased generation of ATP via glycolysis inside β-cells of endocrine pancreas causes the potassium channels to close resulting in an influx of calcium which depolarises the cells and subsequently secretes insulin by the β-cells (Scheepers *et al.,* 2004). Source: Beta Cell Biology Consortium, 2004; (www.betacell.org/content/articleview/article_id/1/).

In patients with type II diabetes the secretion and action of insulin as well as glycaemic control are altered leading to microvascular and macrovascular complications (Donaghue *et al.,* 2009). Diabetic retinopathy, a microvascular complication of type II diabetes mellitus, affects 90% of diabetic patients (Rotimi *et al.,* 2003). Altered glycaemic control causes progressive retinal vessel damage and can ultimately lead to blindness (Cheung *et al.,* 2007; Rotimi *et al.,* 2003). Diabetic nephropathy, another microvascular complication in diabetic patients, causes kidney damage that can result in renal failure (Donaghue *et al.,* 2009). Macrovascular complications of type II diabetes mellitus include coronary heart disease, peripheral vascular disease and stroke (Donaghue *et al.,* 2009). Coronary heart disease is the major cause of death in type II diabetic patients (Hurst and Lee, 2003). Atherosclerosis which causes the stiffening and narrowing of arteries as a result of the formation of lipid plaques in the arterial intima is associated with the development of coronary heart disease, stroke and hypertension in diabetic patients (Southerland *et al.,* 2012).

Although diabetes mellitus and metabolic syndrome have been well investigated and documented using humans and animal models, there still is a need to further investigate ways of preventing

and managing diabetes mellitus, as well as developing experimental models of diabetes mellitus and metabolic syndrome.

2.2 Models of metabolic syndrome

The rise in incidence of the metabolic syndrome in children, adolescents and adults has led to the development of various research models with the aim of investigating the mechanisms involved in the development of metabolic syndrome. The models in use include genetically, chemically and diet-induced rat models (Wu *et al.,* 2007; Russell and Proctor, 2006; Bertram and Hanson, 2001).

2.2.1 Genetic models

Genetic models of components of metabolic syndrome are based on monogenic mutation (Bertram and Hanson, 2001) and include the Zucker diabetic fatty rats (fa/fa), db/db (C57BL/KsJ-db/db) mice, ob/ob (C57BL/6J-ob/ob) mice, Goto-Kakizaki rats and the Otsuka Long-Evans Tokushima fatty rats (Panchal and Brown, 2011; Bertram and Hanson, 2001). The rat models are suitable for evaluating the single gene mutation molecular mechanisms involved in the metabolic syndrome (Panchal and Brown, 2011). In humans metabolic syndrome is a multi-factorial disease therefore monogenic rat models are not suitable in mimicking metabolic syndrome seen in humans (Lehnen *et al.,* 2013; Buettner *et al.,* 2006). There are newer models of metabolic syndrome that are polygenic (Morton *et al.,* 2005). However these polygenic models

are also only suitable for studying the specific gene mutation induced components of metabolic syndrome.

2.2.2 Pharmacologically-induced models

Chemical models of metabolic syndrome are developed using alloxan or streptozotozin (Szkudelski, 2001). Alloxan and streptozotocin are pyramidine glucose analogues which selectively destroy the endocrine pancreatic β-cells (Panchal and Brown, 2011; Lenzen, 2008). The models in which high doses are used are well suited for investigating type I diabetes mellitus, whilst the use of low doses is suited for type 2 diabetes mellitus (Zhang *et al.,* 2008b). In an effort to include the dietary complications, researchers have developed models using a combination of streptozotocin at low doses and high fat or high fructose diets (Wilson and Islam, 2012; Bell *et al.,* 2000).

2.2.3 Diet-induced model

Fat, sucrose and fructose are used as dietary ingredients to induce metabolic syndrome in rat models (Lehnen *et al.,* 2013; Busserolles *et al.,* 2003). High-fructose diet rat models of metabolic syndrome are touted as the best models to use when studying diet-induced metabolic syndrome in humans (Oron-Herman *et al.,* 2008), as they cause the development of diverse complications seen in humans with diet induced metabolic syndrome (Lehnen *et al.,* 2013).

2.2.3.1 Fructose-induced metabolic syndrome: mechanism(s) involved

Fructose is a monosaccharide that requires glucose transporters (Glut 5) to aid its absorption from the jejunum (Miller and Adeli, 2008). Once absorbed from the jejunum, fructose is transported to the liver where fructose-1-phosphatase mediates its metabolism to 3-carbon molecules: glyceraldehyde-3-phosphate and dihydroxyacetone phosphate (Rutledge and Adeli, 2007). These 3-carbon molecules are used to form triglycerides and free fatty acids (Miller and Adeli, 2008). Unlike glucose that activates phosphofructokinase, fructose activates fructose-1-phosphatase (Rutledge and Adeli, 2007). Phosphofructokinase is a rate limiting enzyme that will inhibit formation of triglycerides from glucose, promote glycogen formation from glucose and stimulate insulin secretion (Woo *et al.,* 2010). This means increased absorption of fructose translates into increased lipogenesis (Basciano *et al.,* 2005).

Lipogenesis due to high fructose diet consumption results in increased visceral obesity which promotes the synthesis and release of tumour necrosis factor alpha (TNF-α) and C-reactive protein peptides (Matsudaa and Shimomurab, 2013; Wajchenberg *et al.,* 2009). These inflammatory mediators inhibit Glut-4 transporter protein mRNA formation hence decreasing the overall amount of Glut-4 receptors which are important for insulin-dependent glucose uptake by tissues (Hotamisligil *et al.,* 1994). Decreased Glut-4 receptors lead to inhibition of insulin-activated glucose uptake (Jellinger, 2007), depriving them (cells) of the primary source of energy leading to their dependence on lipids (secondary) and protein (tertiary) energy stores (Hsu *et al.,* 2012). The utilisation of these stores results in elevated plasma triglycerides, free fatty acids and a negative nitrogen balance (Hsu *et al.,* 2012). Increased triglycerides and free fatty acids can over-stimulate

the pancreas causing increased insulin secretion and later on resulting in lipotoxicity of the pancreatic β-cells (Huang *et al.,* 2004). More importantly elevated triglycerides and free fatty acids will lead to increased plasma low density lipoproteins, cholesterol and oxidative stress (Wajchenberg *et al.,* 2009).

Pharmaceutical drugs such as fenofibrate, metformin, statins, thiazolidinediones and sulfonylurea are used to manage the consequences of metabolic syndrome (Florez *et al.,* 2013; Jialal and Smith, 2012).

2.3 Therapeutic interventions

Fenofibrate, one of the many therapeutic drugs used to manage metabolic syndrome, is a hypolipidaemic pharmaceutical drug that lowers cholesterol, triglycerides and low density lipoprotein in patients at risk of developing cardiovascular diseases (Ling *et al.,* 2013). A derivative of fibric acid, it has a half-life of 20 hours (Tsimihodimos *et al.,* 2005) and acts by activating the peroxisome proliferator-activated receptors alpha (PPARα) ligand, thus activating genes that regulate lipoprotein lipase, acyl-Co-A oxidase and carnitine palmitoyltranferase (Jeong *et al.,* 2004b). Lipoprotein lipase promotes the hydrolysis of plasma triglycerides, whilst acyl-Co-A oxidase and carnitine palmitoyltranferase promote β-oxidation of free fatty acids, and results in inhibition of low density lipoprotein formation (Kraja *et al*., 2010; Tsimihodimos *et al.,* 2005). Fenofibrate also lowers cholesterol by preventing its absorption from the small intestinal lumen into the lymph and by promoting HDL formation (Valasek *et al.,* 2007), which (HDL) clears

circulating cholesterol. The use of fenofibrate is approved for children and adolescents and is known to cause similar beneficial and detrimental effects as seen in adults (Singh, 2006; Steinmetz *et al.,* 1981).

While pharmaceutical drugs such as fenofibrate can be used to manage metabolic dysfunction, they are costly and inaccessible to most third world country communities. Traditional medicinal plants including *Terminalia sericea* (*T. sericea*) thus provide valuable, accessible and affordable remedies to 80% of the third world country communities (Fyhrquist *et al.,* 2002).

2.4 *Terminalia sericea*

T. sericea also known as silver cluster-leaf, and locally as vaalboom, mogonono, moxonono, mususu, amangwe, mangwe, mpululu and namatipo (Deutschlander *et al.,* 2009), is widely distributed in sub-tropical Africa (Joseph *et al.,* 2007; Fyhrquist *et al.,* 2002). It (*T. sericea*) contains bioactive compounds and is used in ethnomedicine to treat various systemic ailments in African communities (Eldeen *et al.,* 2006).

2.4.1 Botanical description

T. sericea is a medium sized deciduous tree that grows up to 9m (Chivandi *et al.,* 2013). Its bark is dark grey, thick, fibrous and is longitudinally fissured (Deutschlander *et al.,* 2009). The leaves of *T. sericea* are concentrated on the tips of the shoot and are covered in cottony silver hairs

(Deutschlander *et al.*, 2009). The flowers are a pale-yellow or creamy white colour (Chivandi *et al.*, 2013). The oval shaped *T. sericea* fruits are a purplish-red colour when immature and turn brown when they mature (Chivandi *et al.*, 2013).

Figure 1.2: The leaves and fruit of *T. sericea*

2.4.2 Taxonomical classification

T. sericea belongs to the Kingdom Plantae, Division Magnoliophyta, Class Magnoliopsida, Order Myrtales, Family Combretaceae, Genus *Terminalia* and Species s*ericea* (Anderson and Bell, (1974).

2.4.3 Medicinal uses of *Terminalia sericea*

2.4.3.1 Leaves

Leaves of *T. sericea* are used to treat bacterial infections (Mochizuki and Hasegawa, 2007). Dried leaves are used to treat intestinal infections that cause diarrhoea (Mochizuki and Hasegawa, 2007). In Venda, South Africa, the dried leaf extract of *T. sericea* is used to manage menorrhagia while the dried leaf powder is used to treat infected wounds (Moshi and Mbwambo, 2005; Fyhrquist *et al.,* 2002). A decoction of *T. sericea* plant is used to treat sexually transmitted diseases and virility/infertility in males (Moshi and Mbwambo, 2005; Arnold and Gulumian, 1984).

2.4.3.2 Roots

Hot root infusions are used to treat pneumonia (Deutschlander *et al.,* 2009) and bacterial infections (Mochizuki and Hasegawa 2007). In Tanzania the roots are used to manage headaches, backaches, diabetes mellitus, diarrhoea and gonorrhoea (Moshi and Mbwambo, 2005). In East Africa root decoctions of *T. sericea* have diuretic properties and are used to treat hypertension, bilharziasis and stomach ailments (Fyhrquist *et al.,* 2002).

2.4.3.3 Fruit and bark

The *T. sericea* fruits are used to treat tuberculosis in Malawi (Moshi and Mbwambo 2005; Fyhrquist *et al.,* 2002). The stem bark is used to treat diabetes mellitus (Deutschlander *et al.,* 2009).

2.4.4 Other uses of *Terminalia sericea*

Terminalia sericea extracts rich in sericoside are processed and used to produce skin penetrating topical cream (Moshi and Mbwambo 2005; Rode *et al.,* 2003). Its wood is used as a fuel energy source and building material (Shackleton, 2001). *T. sericea* plants produce edible gum for humans and serve as a source of foraging to browsing herbivores (Aganga *et al.,* 2000; Davidson 1998).

2.5 Chemical composition of *T. sericea*

Different parts of *T. sericea* are composed of various chemical compounds including; phytochemicals, fibre, minerals, vitamins, amino acids and free fatty acids (Chivandi *et al.,* 2013; Nkobole *et al.,* 2011; Davidson, 1998).

2.5.1 Proximate composition of *T. sericea* seed

The *T. sericea* seeds contain 46.2% crude protein and 32.6% lipid (Chivandi *et al.,* 2013). They also contain minerals including; phosphorus (1121.75±10.39 mg.100g^{-1} DM), calcium

(795.20±17.82 mg.100g^{-1} DM) and magnesium (560.70±6.68 mg.100g^{-1} DM) (Chivandi *et al.*, 2013). Chivandi *et al.* (2013) reported that *T. sericea* seeds contain amino acids with glutamic acid (8.07±0.13 g.100g^{-1} DM) being the most concentrated amino acid. The seeds contain 223.30±2.16 g.kg^{-1} DM and 90.27±4.68 g.100g^{-1} DM neutral and acid detergent fibre, respectively (Chivandi *et al.*, 2013).

2.5.2 Lipid profile of *T. sericea* leaves and seeds

T. sericea leaves and seeds contain saturated, monosaturated and polysaturated fatty acids (Chivandi *et al.*, 2013; Davidson, 1998). The most concentrated monosaturated and polysaturated fatty acids in *T. sericea* seed are oleic and α-linolenic, respectively (Chivandi *et al.*, 2013). Whilst total lipid content of *T. sericea* leaves is higher during the dry season than the wet season (Davidson, 1998), the saturated and monoenoic fatty acids are highest during the dry season and oleic and alpha-linolenic fatty acids are highest during the wet and transition season (Davidson, 1998). The presence of linoleic acid in the leaves and seeds of *T. sericea* is suggestive of its potential herbal use as an antioxidant and for the management of metabolic dysfunction (Racine *et al.*, 2010; Zhang *et al.*, 2008a).

2.5.3 Phytochemical constituents

Tannins, triterpernoid saponins and flavonoids are found in the leaves, bark and roots of *T. sericea* (Nkobole *et al.*, 2011; Mochizuku & Hasegawa, 2006; Bessong *et al.*, 2004). The

triterpernoid saponin is made up of sericoside, arjunglucoside and sericic acid (Eldeen *et al.*, 2006; Rode *et al.,* 2003). Anolignan B, termilignan B and arjunic acid are some of the phytochemicals that have been recently isolated in *T. sericea* ethyl acetate extracts (Eldeen *et al.*, 2008). Some of these phytochemicals have been identified as the compounds that give *T. sericea* its antimicrobial activity (Eldeen *et al.,* 2008; Eldeen *et al.,* 2006).

2.5.3.1 Tannins

Tannins are naturally occurring polyphenols found in various fruits, vegetables and plants (Chung *et al.,* 1998), that have antioxidant properties and lower blood glucose and lipid content (Hagerman *et al.,* 1998). They reduce carbohydrate and lipid digestion as well as absorption by inhibiting amylases and lipases, respectively (Chung *et al.,* 1998). Tannins derived from *Terminalia* have been demonstrated to promote wound healing (Chaudhari and Mengi, 2006), decrease visceral fat, lower blood pressure and prevent free radical induced cardiotoxicity (Singh *et al.,* 2008; Dwivedi, 2007); these properties justify the use of *T. sericea* in ethnomedicine to treat diabetes, hypertension and wounds. *In vitro* studies indicated that tannins can cause deterioration of the mucosal lining of the GIT, increase excretion of essential proteins and decrease growth performance in mammals, impair glucose transport and decrease vitamin A absorption in the intestines (Chung *et al.,* 1998). However *in vivo* studies have indicated contradictory results, showing that tannins have no effect on GIT morphology and excretion of essential amino acids (Chung *et al.,* 1998).

2.5.3.2 Saponins

Saponins are glycosides that occur naturally in plants and form soap-like foam in water (Wina *et al.,* 2005). Triterpenoid saponins, namely lupeol, resveratrol-3-o-B-rutinoside, resveratrol, stigmasterol, B-sitoster have been extracted from *T. sericea* root and bark extracts (Nkobole *et al.*, 2011; Joseph *et al.,* 2007; Moshi & Mbwambo, 2005). Saponins decrease blood glucose, cholesterol and low density lipoprotein concentration in humans (Mahato, 1988). It is hypothesised that *T. sericea* derived saponins lower blood glucose and cholesterol content by stimulating the endocrine pancreatic β-cells to secrete insulin and by inhibiting glucose and cholesterol absorption from the GIT (Subramaniam *et al.,* 2011**;** Francis *et al.,* 2002). Saponins have been demonstrated to inhibit glucose uptake in Ehrlich ascites tumour cells (Hasegawa 1994) and thus inhibit growth of tumour cells, probably by depriving them of nutrients (Francis *et al.,* 2002). Furthermore, saponins have scavenging properties that allow them to protect cells and cell membranes from damage by free radicals (Eckel *et al.,* 2005). The presence of saponins in *T. sericea* leaves is a probable indicator of its' extracts to be used in the management of metabolic dysfunction. Saponins of *T. sericea* have been demonstrated to have anti-inflammatory activity (Eldeen *et al.,* 2005). Treatment of hypophysial cells with saponins led to increased production of luteinising hormone by the cells (Francis *et al.,* 2002). The latter increases production of testosterone by testes which is responsible for the development secondary male sex characteristics hence this may justify the use of *T. sericea* in ethnomedicine to treat virility/infertility men.

2.5.3.3 Flavonoids

Flavonoids are polyphenolic compounds found in plants (Tapas *et al.,* 2008) and are well known for their antioxidant activity. Flavonoids, namely rutinoside, catechin, epicatechin, gallocatechin and epigallocatechin have been extracted from *T. sericea* stem bark (Nkobole *et al.,* 2011). Free radicals that are bound to flavonoids cannot cause cellular damage (Sandhar *et al.,* 2011). The antioxidant and anticoagulant properties of flavonoids prevent vessel endothelial dysfunction, thrombosis and cardiovascular diseases (Sandhar *et al.,* 2011). Flavonoids stimulate insulin release and pancreas β-cells regeneration (Tapas *et al.,* 2008). They also promote hepatocyte regeneration by increasing activity of RNA polymerase I (Tapas *et al.,* 2008), thus suggesting that they have potential to treat liver disease. Additionally, flavonoids are cyclooxygenase (COX) inhibitors and they can potentially be used as anti-allergy and anti-inflammatory compounds (Tapas *et al.,* 2008).

2.6 Pharmacological actions of *T. sericea* extracts

2.6.1 Antimicrobial activity

Triterpene saponins, tannins and flavonoids are toxic to microorganisms; they bind to microbial cell membranes and impair their functioning (Bowyer *et al.,* 1995; Scalbert, 1991). Both organic and aqueous decoctions of *T. sericea* exhibit antimicrobial activity (Eldeen *et al.,* 2008; Eldeen *et al.,* 2006; Moshi and Mbwambo, 2005; Fyhrquist *et al.,* 2002). *T. sericea* organic and aqueous root, stem bark and leaf extracts, *in vitro* exhibit anti-bacterial activity against *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Enterobacter aerogenes, Bacillus*

subtilis, Micrococcus luteus, Escherichia coli, Klebsiella pneumonia and *Sarcina specie* (Eldeen *et al.,* 2008; Eldeen *et al.,* 2006; Moshi and Mbwambo, 2005; Fyhrquist *et al.,* 2002). They also exhibit antifungal activity against *Candida albicans, Aspergillus niger, Aspergillus fumigates, Cryptococcus neoformans, Microsporum canis* and *Sporothrix schenckii* (Eldeen *et al.,* 2006; Moshi and Mbwambo, 2005; Masoko *et al.,* 2005; Steenkamp *et al.,* 2004; Fyhrquist *et al.,* 2002). The antimicrobial activity of *T. sericea* extracts is said to be due to the presence of triterpene saponins, tannins, flavonoids and anolignan B (Eldeen *et al.,* 2006; Fyhrquist *et al.,* 2002).

2.6.2 Anti-HIV activity

Tannins, flavonoids, triterpene saponins, anolignan A and anolignan B are thought to be the compounds that give *T. sericea* its anti-HIV properties (Tshikalange *et al.,* 2008; Wall *et al.,* 1996). *In vitro* studies have reported that organic and aqueous *T. sericea* leaf and root extracts have inhibitory properties against human immunodeficiency virus (HIV-1) replication enzymes (Tshikalange *et al.,* 2008; Bessong *et al.,* 2004).

2.6.3 Antioxidant activity

Studies have reported that *T. sericea* aqueous and methanolic stem bark extracts have antioxidant activity (Nkobole *et al.,* 2011; Steenkamp *et al.,* 2004). *T. sericea* aqueous and methanolic bark extracts significantly lowered oxidant production in formyl-methionyl-leucyl-phenylalanine

stimulated neutrophils (Steenkamp *et al.,* 2004). The antioxidant properties of *T. sericea* stem bark extracts are said to be due to triterpene saponins tannins and flavonoids, which scavenge harmful oxidants (Nkobole *et al.,* 2011; Eckel *et al.,* 2005).

2.6.4 Antidiabetic and lipolytic activity

Nkobole *et al.* (2011) tested the anti-diabetic activity of *T. sericea* stem bark by measuring inhibitory activity of alpha-glucosidase and alpha amylase in an *in vitro* bioassay. *T. sericea* stem bark extracts showed strong inhibition of alpha-glucosidase and alpha-amylase, thus suggesting that it may have anti-diabetic properties. Similarly, Tshikalange *et al*. (2008) reported inhibitory activity *T. sericea* chloroform, ethyl-acetate and 70% acetone bark extracts on alpha-glucuronidase and beta-glucuronase. However, an *in vivo* study by Moshi and Mbwambo (2005) reported that administering a single dose (200 $mg.kg^{-1}$ body mass) of 20% aqueous-ethanol *T. sericea* root extracts had no effect on glucose tolerance of Wistar mice.

2.6.5 Anti-inflammatory

Eldeen *et al.* (2005) reported that ethyl acetate, ethanol and aqueous root and stem bark extracts of *T. sericea* inhibited cyclooxygenase-1, an enzyme key to inflammatory responses. Central obesity can induce an inflammatory response that results insulin resistance (Hotamisligil *et al.,* 1994). Mochizuki and Hasegawa (2007) tested *T. sericea* roots for their protective effect against 2,4,6-trinitrobenzesulfonic acid (TNBS) induced inflammatory bowel disease (IBD). The *T.*

sericea sericoside in the roots of *T. sericea* was reported to have protective effects against TNBS induced IBD (Mochizuki and Hasegawa, 2007). Additionally, flavonoids and saponins, present in *T. sericea* extracts have been reported to mitigate inflammation by suppressing mediators of inflammation (Eldeen *et al.,* 2008; Eldeen *et al.,* 2006; Eldeen *et al.,* 2005).

2.6.6 Antitumour effect

T. sericea leaf and root extracts have been shown to be cytotoxic to T 24 bladder cancer cell, Hela cervical cancer cells and MCF breast cancer cells and thus inhibiting their proliferation (Fyhrquist *et al.,* 2006; Bessong *et al.,* 2004). Tannins, saponins and flavonoids, which are present in *T. sericea* extracts, have been implicated as being anti-cancerous agents (Fyhrquist *et al.,* 2006; Bessong *et al.,* 2004).

2.6.7 Toxicity

Plants contain phytochemicals that have the potential to be toxic. It is important to evaluate the toxic potential carried by traditional plants. Cytotoxicity of *T. sericea* dichloromethane, ethyl acetate, dichloro:methanol, methanol and ethanol root extracts, have been investigated using standard brine shrimp toxicity tests. It was reported that all extracts showed toxicity but dichloromethane and petroleum extracts being the exceptions (Moshi and Mbwambo, 2005). Bessong *et al.* (2004) reported that *T. sericea* aqueous and methanolic extracts showed no toxicity in Hela P4 cell lines that were treated with 600 $\mu g.ml^{-1}$ of the extracts.

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CHAPTER 3: MATERIALS AND METHODS

3.0 Source of *T. sericea* leaves

T. sericea leaves were collected in the summer of 2012 at Maubane N1 toll road, Republic of South Africa. The area on latitude 25° 16' 34.13", longitude 28° 17' 55.92" has an average annual maximum temperature of 27ºC, mean annual rainfall of 530 mm-650 mm (M'marete, 2003). The area (Maubane) is characterised by black clay soils (Coetzee *et al.*, 2003).

The identification of the collected leaves was authenticated by a botanist (Mr Donald McCallum) at the University of the Witwatersrand's Herbarium. A sample of the *T. sericea* plant was stored at the University of the Witwatersrand Herbarium under the reference number J96241.

3.1 Preparation of *T. sericea* leaf extracts

The leaves were dried overnight in an oven (Salvis ®, Salvis Lab, Switzerland) at 40°C (Ahmed *et al.*, 2005), after which they were ground to a course powder in a blender (Waring®, Lasec SA, Johannesburg, South Africa). The extraction was done using distilled water. Briefly, every 25 g of the leaf powder was mixed with 250 ml of boiling distilled water and placed on a shaker for 24 hours (Bessong *et al.*, 2004). The mixture was filtered twice: firstly through a cheese cloth and thereafter through filter paper (Whatmann®, No 1 size 50 mm, England) (Bessong *et al.*, 2004). The resultant filtrate was concentrated by evaporating at 50°C in a rotor vacuum evaporator [Labocon (Pty) Ltd, Krugersdorp, South Africa] and then placed in an oven (Salvis ®, Salvis Lab,

Switzerland) at 40°C till they reached dryness after four days. The dry extracts were stored in air tight dark glass bottles and kept in a dark cupboard until use.

3.2 Determination of the phytochemical composition of *T. sericea* leaf extracts

Qualitative tests were used to determine the presence and or absence of flavonoids, terpenoids, saponins, tannins and anthraquinones in *T. sericea* aqueous leaf extracts.

3.2.1 Flavonoids

The presence of flavonoids was determined as described by Doss (2008). Briefly, 2 g of *T. sericea* leaf filtrate was added to 5 ml of 10% ammonium hydroxide in a test tube. Then 1ml of concentrated sulphuric acid was added to the mixture. Presence of flavonoids was indicated by the development of a yellow colour.

3.2.2 Terpenoids

The presence of terpenoids in the *T. sericea* leaf extract was determined as described by Ayoola *et al*. (2008). Briefly, 0.5 g of the *T. sericea* leaf extract was added to 2ml chloroform in a test tube following which 3ml of concentrated sulphuric acid was added to the mixture. Development of a reddish brown colour in the interface layer indicated the presence of terpenoids.

3.2.3 Saponins

The presence of saponins in the T. sericea leaves was determined as described by Ayoola *et al.* (2008). Briefly, 1 ml of *T. sericea* leaf resultant filtrate was added to 10 ml distilled water in flask and shaken for 30 seconds. Frothing confirmed the presence of saponins.

3.2.4 Tannins

The presence of tannins in the *T. sericea* leaves was determined as described by Ayoola *et al*. (2008). Briefly, 0.5 mg of *T. sericea* extract was boiled in 5 ml of distilled water in a beaker. Ferric chloride was then added. Development of a dark purple black colour showed the presence of tannins.

3.2.5 Anthraquinones

The presence of anthraquinones in the *T. sericea* leaves was determined as described by Ayoola *et al*. (2008). Briefly 5 g of *T. sericea* extract was boiled in 10 ml 2M sulphuric acid. The mixture was then filtered; the resultant filtrate was added to 5 ml chloroform to form two layers. The chloroform layer was then carefully pipetted into a test tube containing 1ml of diluted ammonium hydroxide and colour changes were observed.

3.3 Brine shrimp toxicity assay

3.3.1 Hatching of nauplii

Cysts of *Artemia salina* were hatched at room temperature in saline water (2% NaCl) overnight. The saline was aerated by bubbling atmospheric air in the hatching container using a 60ml syringe connected to an infusion pump (Harvard Apparatus, MA, USA).

3.3.2 Brine shrimp assay

Fifty milligrams (50 mg) of the *T. sericea* aqueous leaf extracts were dissolved in 5ml dimethylsulfoxide and made to 100ml with 2.9% saline solution. The extract solution was serially diluted to concentrations of 5000, 500, 50, 5, 0.5 and 0.05 $\mu g.ml^{-1}$. The extract solutions (2ml) were transferred in duplicate to a 24-well plate. Saline solution (2.9%) was used as a negative control. Potassium dichromate, which is toxic to brine shrimp, was used as a positive control at concentrations of 3000, 300, 30 and 3 $\mu g.ml^{-1}$. The freshly hatched nauplii were placed in the wells (10 per well) followed by incubation at room temperature for 24 hours. The numbers of dead nauplii were then counted percentage mortality determined and the half maximal lethal concentration (LC_{50}) calculated.

3.4 Ethical clearance for use of animals and the study site

The study, conducted in the Central Animal Services (CAS) unit at the Faculty of Health Sciences, University of the Witwatersrand, was approved by the Animal Ethics Screening Committee (AESC) of the University of the Witwatersrand, Johannesburg, South Africa (AESC number: 2012/29/05). Tissue assays were done in appropriate laboratories of the School of Physiology, University of the Witwatersrand.

3.5 Animals: feeding and housing

Forty 21-day weanling male Wistar rat pups were used in the experiment. The commercial rat chow used in this study was bought from EPOL Animal Feed Manufacturers [Epol®, Centurion, South Africa (protein 170 $g.kg^{-1}$, fat 25 $g.kg^{-1}$, fibre 70 $g.kg^{-1}$, calcium 25 $g.kg^{-1}$, phosphorus 6 $g.kg^{-1}$ and total lysine 6.5 $g.kg^{-1}$)]. The pups had *ad libitum* access to a standard rat chow. Each pup was individually housed in a perspex cage with clean wood shavings for bedding. Bedding was changed once a week. Room temperature was maintained at 22±2°C with lights on between 7am to 7pm. The pups were given 3 days familiarisation period before the start of the experimental period.

3.6 Treatment doses and drug vehicle medium preparation

The dosage of fenofibrate used in the current study was similar to that used by Legendre *et al.* (2002). The low and high dose of the aqueous *T. sericea* leaf extracts were similar to those used by Njomen *et al.* (2009) and Rao and Nammi (2006). The 2 ml gelatine cubes used as vehicle mediums for *T. sericea* aqueous leaf extract and administration were prepared as described by Kamerman *et al.* (2004). Briefly, 8g of Sheridans, clear, unflavoured edible gelatine [Retailer Brands (Pty) Ltd, South Africa], 17 g brown sugar (Sunsweet® Huletts, South Africa) and 5ml Beefy Bovril (Bokomo Foods, South Africa) were added to 100ml of warm water and then the solution was allowed to cool down and form solid moulds. The Fructose solution was prepared by adding commercial fructose (Huletts®, Fructose Concentrated Sweetness, Low GI, South Africa) to a litre of warm water in a beaker. Food Colouring [Robertsons, Red Food colouring, Retailer Brands (Pty) Ltd, South Africa] was added to the fructose solution to help with identification.

3.7 Treatments and experimental design

Forty 21-day old male Wistar pups were randomly allocated to five treatment regimens. Each treatment regimen had 8 pups. The pups had *ad libitum* access to a standard rat chow. Group 1 pups were given plain gelatine cubes and plain drinking water (PC + PW), group 2: plain gelatine cubes and 12% fructose solution (PC + FS), group 3: gelatine cubes containing fenofibrate at a concentration calculated to deliver 100 $mg.kg^{-1}$ body mass per day and 12% fructose solution (FF + FS), group 4: gelatine cubes containing low dose of the *T. sericea* extract at a concentration calculated to deliver 100 $mg.kg^{-1}$ and 12% fructose solution (LD + FS) and

group 5: gelatine cubes containing high dose of the *T. sericea* extract at a concentration calculated to deliver 400 $mg.kg^{-1}$and 12% fructose solution (HD + FS). The pups were maintained on the treatment regimens for 12 weeks.

3.8. Body mass measurement

The rats were weighed (Snowrex Electronic Scale, Clover Scales, Johannesburg) twice per week to monitor growth performance. The body mass measurements also allowed for the maintenance of a constant dosage of the *T. sericea* leaf extract and fenofibrate relative to body mass over the 12-week treatment period.

3.9. Experimental intervention and terminal procedures

3.9.1 Oral glucose tolerance test

After 12 weeks of treatments the rats were given a 12-hour overnight fasting period before the determining their tolerance to an oral glucose challenge. Fasting blood glucose concentrations were determined (time interval 0) on each rat using a glucometer calibrated according to manufacturer' instructions (Ascentia, Elite^{TM}, Bayer Corporation, Mishawaka, USA). Blood for the fasting blood glucose concentration determination and after an oral glucose challenge was taken via a pin prick of the tail vein after sterilisation of the area to be pricked with a cotton gauze swab impregnated with alcohol (Loxham *et al.*, 2007). Following the determination of the

fasting blood glucose concentrations, rats were gavaged via orogastric intubation with 2g kg^{-1} body mass of sterile 50% (w/v) D-(+)-glucose solution [Merck Chemicals (Pty) Ltd, Johannesburg, South Africa]. Post gavage glucose concentrations at time intervals 15, 30, 60, 120 and 180 minutes, were then determined as previously described (Leng *et al.*, 2004).

3.9.2 Terminal procedures

Following the OGTT, the rats were put back onto their respective treatments for 48 hours. Thereafter, they were fasted overnight and their fasting blood glucose levels were then determined (the following morning), using a calibrated glucometer (Ascensia Elite^{TM} Blood glucose meter, Bayer Corporation, Mishawaka, USA) according to the manufacturer's instructions. The rats were then euthanased by intra-peritoneal injection with an overdose of sodium pentobarbitone (Eutha-naze, Bayer, Johannesburg, South Africa) at 200 $mg.kg^{-1}$ body mass.

3.9.2.1 Blood collection, processing and plasma storage

Following euthanasia of the rats, 8ml of blood was collected via cardiac puncture using 21G needles and 10ml syringes and divided equally into 4ml plain and 4ml heparin blood tubes (Becton Dickinson VACUTAINER Systems Europe, Meylan Cedex, France). A drop of the blood was then used to determine fasting blood triglycerides using a calibrated Accutrend triglyceride meter (Roche, Mannheim, Germany) according to the manufacturer's instructions. The blood samples were spun for 15 min at 5000 G at 20 ^{0}C in a Sorvall RT® 6000B centrifuge

(Pegasus Scientific Inc., Rockville USA). The plasma and serum were collected and then stored at -20 ^{0}C for the analysis of insulin and general health profile markers, respectively.

3.9.2.2 Determination of visceral organ morphometry

Following blood collection, the abdomen was cut open by midline incision. The liver, stomach, pancreas, small and large intestines, ceacum, visceral fat, epididymal fat, heart and kidney were carefully dissected out. The contents of the stomach, small and large intestines and caecum were gently emptied after which the GIT and other viscera were weighed on a Presica 310M electronic balance (Presica Instruments AG, Switzerland). The small and large intestines were gently stretched out on a board and their lengths were measured using a ruler mounted on a cooled dissection board. A sample of the liver was stored at -20ºC for determination of lipid and glycogen content. A sample of the liver and small intestine (proximal and distal) were preserved in 10% phosphate buffered formalin for histology analysis. The remainder of the liver samples were frozen for the determination of liver lipid and glycogen content. The masses of the empty carcasses were then determined after.

3.9.2.3 Determination of indexes of long bone growth

The femoral attachment of left hind leg to the pelvis was excised from each of the carcasses, defleshed and disarticulated from the tibia. The bones were dried in an oven (Salvis ®, Salvis Lab, Switzerland) at 50^{o}C for 5 days and then weighed after which the tibia (measured between tibia head medial malleolus) and femur (measured between distal femoral articular surface to the

greater trochanter) lengths were measured with a pair of vernier calipers (Hi-impact, Dejuca, South Africa). Bone density was then caluclated using the formula:

Bone density = mass of bone (mg) /length of bone (mm)

To further evaluate bone density of tibiae and femora, radiographs were taken using a Fuji film X-ray machine (Industrial X-ray film FR, Fuji Photo Film Co, Ltd, Tokyo, Japan). Briefly, the bones were placed on the photographic plate at a distance of 1 metre from the X-ray light source with settings of 4.8 kVp, 0.71 mA per plate was used.

3.9.2.4 Determination of liver lipid and glycogen storage

Liver lipids were extracted as described by Bligh and Dyer (1959). Briefly, 5g of liver was steeped overnight in 150 ml of a 2:1 mixture of chloroform methanol at 4 ^{o}C. The mixture was filtered through filter paper (Whatmann®, No 1, size 185 mm, pore size 7-11µm, England) into 250ml separating funnel. Then 30ml of 0.9% saline was added to the filtrate and mixed thoroughly. The resultant mixture was allowed to stand overnight in a refrigerator at 4^{o}C after which the organic layer was separated out into round bottomed flask. The organic aliquot was reduced to dryness under vacuum at 37^{o}C. The lipid extract was dissolved in 20ml chloroform and 2 ml of the extract mixture was dried to determine the lipid content in the liver.

Liver glycogen was determined indirectly by acid hydrolysis to glucose and measurement of glucose (Passonneau and Lauderdale, 1974). The glucose concentration of the hydrosylate was determined on an Accu-Chek Active glucometer (Roche, Germany).

3.9.2.5 Determination of liver and small intestine histomorphometry

The preserved liver and small intestine samples were embedded in paraffin wax, sectioned, and then stained with haematoxylin and eosin, on a glass slide and covered with a glass cover slip (Reyes-Gordillo *et al.,* 2007). The slides were then viewed under a light microscope using an eye piece micrometer (Reichert®, Austria). The height of villi and crypt depth of the small intestines were measured. The hepatocyte size and cell numbers within a linear field (100μm) was measured at high power magnification of 400X.

3.9.2.6 Determination of biochemical health profile markers

Serum concentrations of ALT, ALP, urea nitrogen, creatinine, total protein, albumin, globulin, total bilirubin, phosphorus, calcium, cholesterol and amylase of the rats were determined using a colorimetric-based clinical chemistry analyzer (IDEXX VetTest® Clinical Chemistry Analyser, IDEXX Laboratories Inc., USA) as per the manufacturer's instructions. Briefly, each stored serum sample was thawed and allowed to warm to room temperature, gently inverted to mix the contents and then placed into the analyzer which automatically drew up 150 μL of the serum. Ten microlitres (10 μL) of serum were then loaded onto each of the pre-loaded disks after which each sample was then analysed and print outs provided.

3.9.2.7 Plasma insulin concentration determination

Plasma insulin was determined by enzyme linked immunosorbent assay (ELISA) using a Rat insulin kit (DRG ®, Rat Insulin High Range, USA) according to the manufacturer's instructions. The assay employed a quantitative sandwich enzyme immunoassay technique which utilizes a monoclonal antibody specific for rat insulin. Absorbencies were read at 450 nm using a plate reader (Multiskan Ascent, Lab system, model nº 354, Helsinki, Finland). A standard curve was constructed using calibrator concentration sand the concentrations of insulin in the samples were determined. A detailed description of the protocol employed is described in appendix 2.

3.9.2.8 Computation of the HOMA IR index

The HOMA-IR used for the assessment of insulin resistance, was computed as shown in the equation below:

HOMA-IR = [fasting insulin ($\mu U.ml^{-1}$) × fasting glucose ($mmol.l^{-1}$)/22.5] (Matthews *et al.*, 1985)

3.10 Statistical analysis

All data are expressed as mean ± SD. Data analysis was done using Graphpad Prism 5 software (Graph-pad Software Inc., San Diego, USA). Statistical significance was considered when $P \leq 0.05$. Data for weekly body mass and OGTT were analysed using a repeated measures analysis of variance while data for other parameters were analysed by a one-way analysis of variance. The

Bonferroni post hoc test was used to compare means. The model used for the analysis of variance for weekly body masses and the glucose tolerance test was:

$Y_{ijk} = \mu + T_i + B_j + C_k + e_{ijk}$; where;

Y_{ijk} = blood glucose concentration at time C post gavage

μ = overall mean common to all observations

T_i = effect of treatment (n = 1,2,..5)

B_j = fixed effect of individual rat (1,2,3.....40)

C_k = fixed effect of sampling time on blood glucose concentration (= 1,2.....6)

e_{ijk} = residual random error

The model used for analysis of variance for variables determined at study termination was:

$Y_{ijk} = \mu + T_i + B_j + e_{ijk}$; where;

Y_{ijk} = response variable of interest

μ = overall mean to all observations

T_i = effect of treatment (n = 1,2,..5)

B_j = fixed effect of individual rat (1,2,3.....40)

e_{ijk} = residual random error

CHAPTER 4: RESULTS

4.1 Phytochemical composition of aqueous leaf extract

Table 4.1 shows the phytochemical composition of the aqueous *T. sericea* leaf extract.

Table 4.1 Phytochemical constituents of the aqueous *T. sericea* leaf extract

Phytochemical	Present/Absent
Terpernoids	Present
Saponins	Present
Flavonoids	Present
Tannins	Present
Anthraquinones	Absent

The aqueous *T. sericea* leaf extracts contained terpernoids, saponins, flavonoids, and tannins but did not appear to contain anthraquinones.

4.2 Brine shrimp toxicity assay

Figure 4.1 shows the results of the toxicity test of aqueous *T. sericea* leaf extracts on brine shrimp nauplii.

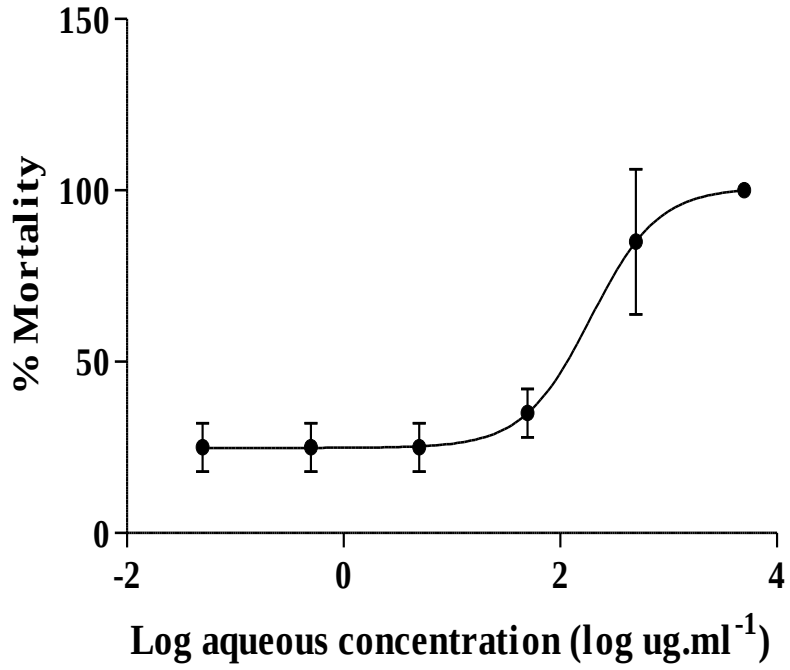

Figure 4.1: Toxicity of aqueous *T. sericea* extracts on *Artemia salina* (brine shrimp) nauplii.

The aqueous *T. sericea* leaf extracts had a median lethality concentration of (LC_{50} - 24 hours) of 97.96±0 µg.ml^{-1}.

While the median lethality concentration of (LC_{50} - 24 hours) of aqueous *T. sericea* leaf extracts was 97.96±0 $\mu g.ml^{-1}$ (Figure 4.1). The LC_{50} - 24 hours was 3 $\mu g.ml^{-1}$ for the positive control; potassium dichromate (data not shown).

4.3 Mortality and morbidity of the rats

During the *in vivo s*tudy, all the rats remained healthy with no incidental or iatrogenic rat mortalities recorded.

4.4 Effects of aqueous *T. sericea* leaf extract on growth performance

4.4.1 Body mass and empty carcass mass

Figures 4.2A and 4.2B show the induction and terminal body masses and empty carcass masses, respectively, of the rats across the different treatment regimens.

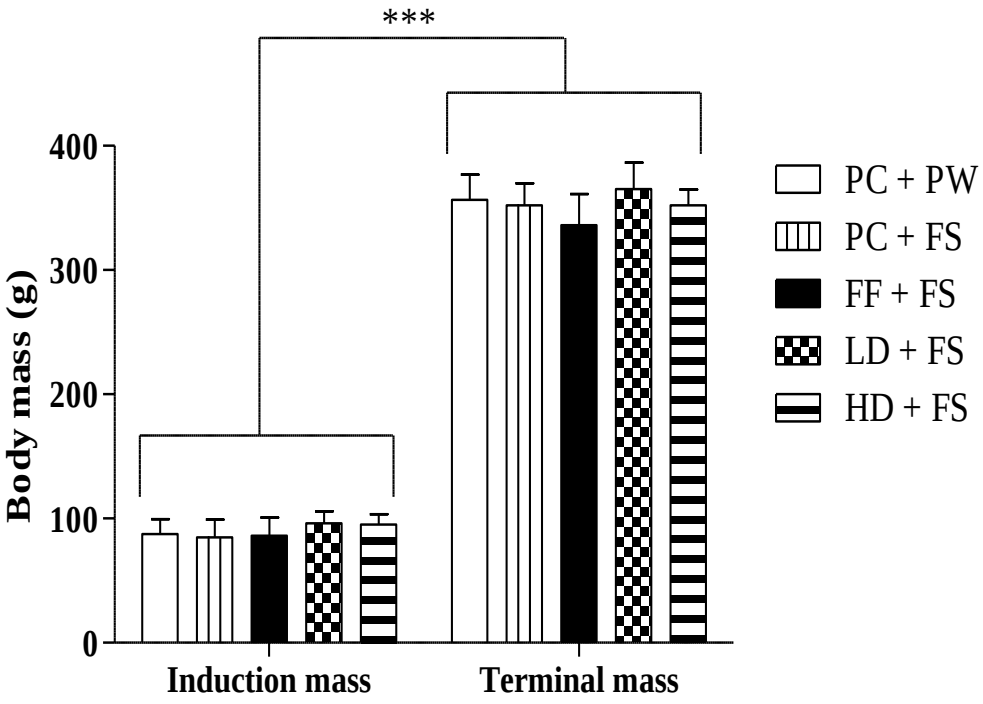

Figure 4.2A: The induction and terminal body masses of the rats given the different treatments.

*** $P < 0.0001$. The induction body masses of the rats were statistically similar ($P > 0.05$) across the different treatments. The terminal body masses of the rats were similar ($P > 0.05$) across the different treatments. Rats in all groups grew significantly ($P < 0.0001$) compared to their induction mass. PC + PW = plain gelatine cube + plain water; PC + FS = plain gelatine cube + 12% fructose solution; FF + FS = 100 $mg.kg^{-1}$ body mass per day fenofibrate + 12% fructose solution; LD + FS = low dose aqueous *T. sericea* leaf extract (100 $mg.kg^{-1}$ body mass per day) + 12% fructose solution; HD + FS = high dose aqueous *T. sericea* leaf extract (400 $mg.kg^{-1}$ body mass per day) + 12% fructose solution. Data presented as mean±SD; n = 8 per treatment.

While the induction and terminal body masses of the rats across the treatments were similar ($P > 0.05$), rats given each treatment grew significantly ($P < 0.0001$) compared to their induction body masses (Figure 4.2A)

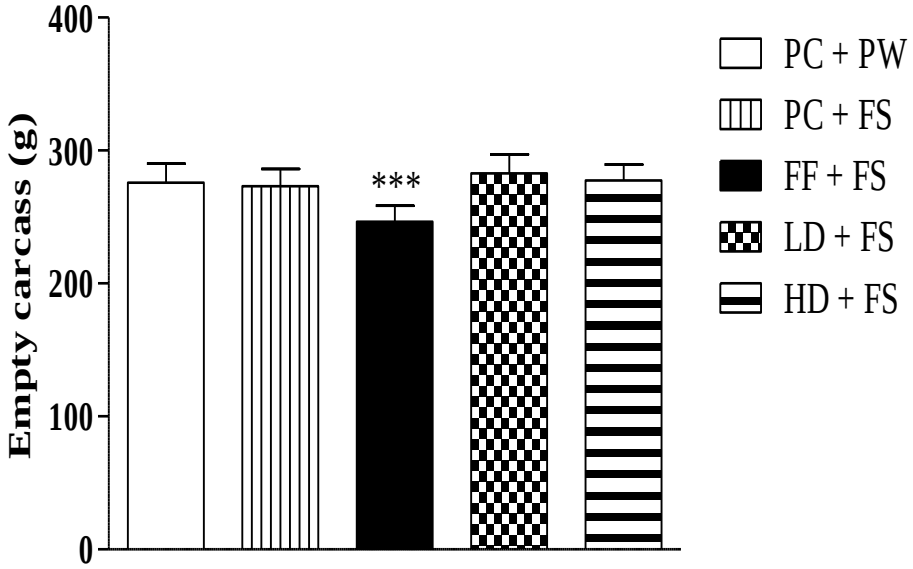

Figure 4.2B: Effect of aqueous *T. sericea* leaf extract on the empty carcass mass of the rats.

*** $P < 0.0001$. Rats given FF + FS had significantly ($P < 0.0001$) lighter empty carcass masses compared to empty carcass masses of rats from all treatment groups. Rats given PC + PW, PC + FS, LD + FS and HD + FS, respectively, had similar empty carcass mass. PC + PW = plain gelatine cube + plain water; PC + FS = plain gelatine cube + 12% fructose solution; FF + FS = 100 $mg.kg^{-1}$ body mass per day fenofibrate + 12% fructose solution; LD + FS = low dose aqueous *T. sericea* leaf extract (100 $mg.kg^{-1}$ body mass per day) + 12% fructose solution; HD + FS = high dose aqueous *T. sericea* leaf extract (400 $mg.kg^{-1}$ body mass per day) + 12% fructose solution. Data presented as mean±SD; n = 8 per treatment.

While the empty carcass masses of rats given FF + FS were significantly ($P < 0.0001$) lower compared to the carcass masses of all rats from other treatment groups, rats given PC + PW, PC + FS, LD + FS and HD + FS, that had similar ($P > 0.05$) empty carcass masses (Figure 4.2B).

4.4.2 Linear growth

Table 4.2 shows lengths, masses and densities of tibiae and femora of the rats following administration of their respective treatments for 12 weeks.

Table 4.2: Effect of aqueous *T. sericea* leaf extract on tibiae and femora lengths, masses and density of the rats

	PC + PW	PC + FS	FF + FS	LD + FS	HD + FS	Significance level
Tibia						
Length (mm)	40.74±0.66[a]	40.51±0.80[a]	39.65±0.71[b]	40.92±0.66[a]	40.56±0.65[a]	*
Mass (mg)	577.75±31.43[ab]	573.00±35.92[ab]	551.75±26.24[a]	598.5±28.08[b]	585.62±24.68[ab]	**
Density ($mg.mm^{-1}$)	14.18±0.61[a]	14.15±0.90[a]	13.91±0.57[a]	14.62±0.53[a]	14.43±0.53[a]	ns
Femur						
Length (mm)	36.45±0.97[a]	35.65±1.70[a]	35.48±0.93[a]	36.34±0.24[a]	37.01±1.55[a]	ns
Mass (mg)	676.00±36.21[a]	671.5±37.83[a]	653.3±48.26[a]	698.8±21.11[a]	686±32.52[a]	ns
Density ($mg.mm^{-1}$)	18.54±0.71[a]	18.86±1.08[a]	18.40±0.93[a]	19.23±0.55[a]	18.55±0.85[a]	ns

n.s. = not significant, $P > 0.05$. *$P \leq 0.05$; **$P < 0.01$. [ab]Within row means with different superscripts are significantly different at $P \leq 0.05$. Rats given FF + FS had significantly ($P = 0.04$) shorter tibiae compared to the tibiae lengths of rats from all treatment groups. Rats given LD + FS had significantly ($P = 0.0024$) heavier tibiae compared to that of rats given FF + FS. Femora lengths and tibiae and femora densities of the rats across the different treatments were similar ($P > 0.05$). PC + PW = plain gelatine cube + plain water; PC + FS = plain gelatine cube + 12% fructose solution; FF + FS = 100 $mg.kg^{-1}$ body mass per day fenofibrate + 12% fructose solution;

LD + FS = low dose aqueous *T. sericea* leaf extract (100 mg.kg^{-1} body mass per day) + 12% fructose solution; HD + FS = high dose aqueous *T. sericea* leaf extract (400 mg.kg^{-1} body mass per day) + 12% fructose solution. Data presented as mean±SD; n = 8 per treatment.

The rats that received FF + FS had significantly ($P = 0.04$) shorter tibiae compared to rats from all other treatment groups, which (PC + PW, PC + FS, LD + FS and HD + FS) had similar ($P > 0.05$) tibiae lengths (Table 4.2). While the rats given FF + FS had significantly ($P = 0.0024$) lighter tibiae compared to their counterparts given LD + FS, rats given other treatments (PC + PW, PC + FS and HD + FS) had similar ($P > 0.05$) tibiae masses (Table 4.2). Femora lengths and masses as well as tibiae and femora densities were similar ($P > 0.05$) across treatment groups (Table 4.2).

Figure 4.3 shows radiographs of femora and tibiae of the rats after 12 weeks on their respective treatments.

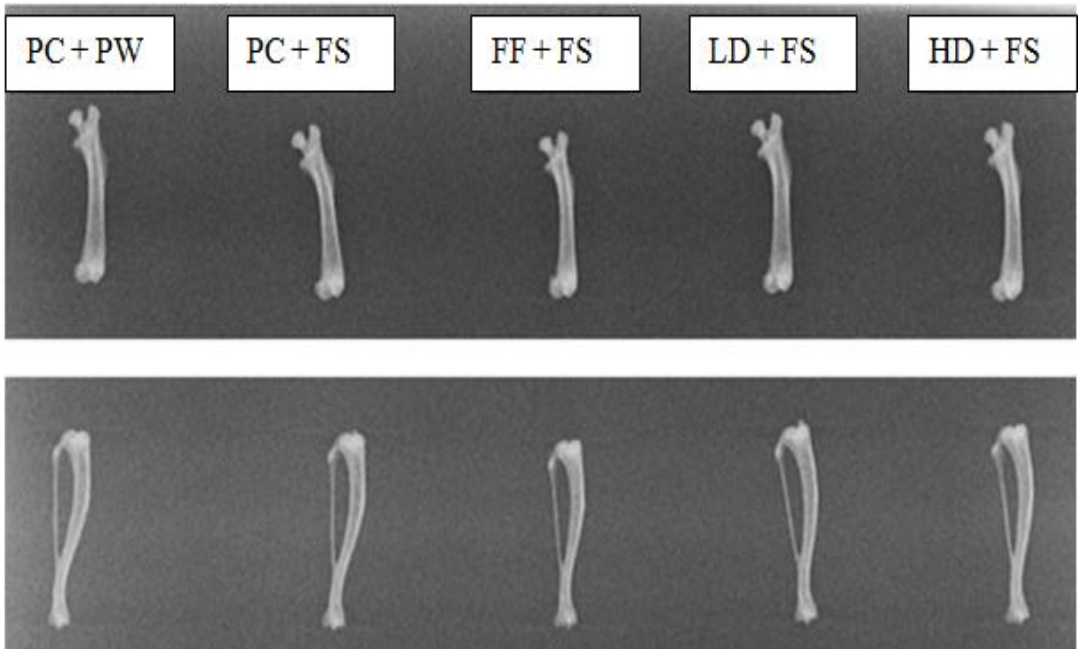

Figure 4.3: Radiograph images of femora and tibiae of the Wistar rats.

The top row shows radiographs of the femora of rats from different treatment regimens and the bottom row shows the tibiae of representative rats from the different treatment groups. PC + PW = plain gelatine cube + plain water; PC + FS = plain gelatine cube + 12% fructose solution; FF + FS = 100 mg.kg $^{-1}$ body mass per day fenofibrate + 12% fructose solution; LD + FS = low dose aqueous *T. sericea* leaf extract (100 mg.kg^{-1} body mass per day) + 12% fructose solution; HD + FS = high dose aqueous *T. sericea* leaf extract (400 mg.kg^{-1} body mass per day) + 12% fructose solution.

There were no observable differences in the radiographical densities of the bones across treatment regimens (Figure 4.3).

4.5 Effect of aqueous *T. sericea* leaf extract on glucose tolerance

Figures 4.4A shows the blood glucose concentrations at basal (0 min) and at 15, 30, 60, 120 and 180 minutes post gavage with 50% percent glucose solution at 2 $g.kg^{-1}$ body mass of the rats following 12 weeks on their respective treatment regimens. Figure 4.4B shows the area under the curve calculated from oral glucose tolerance test results.

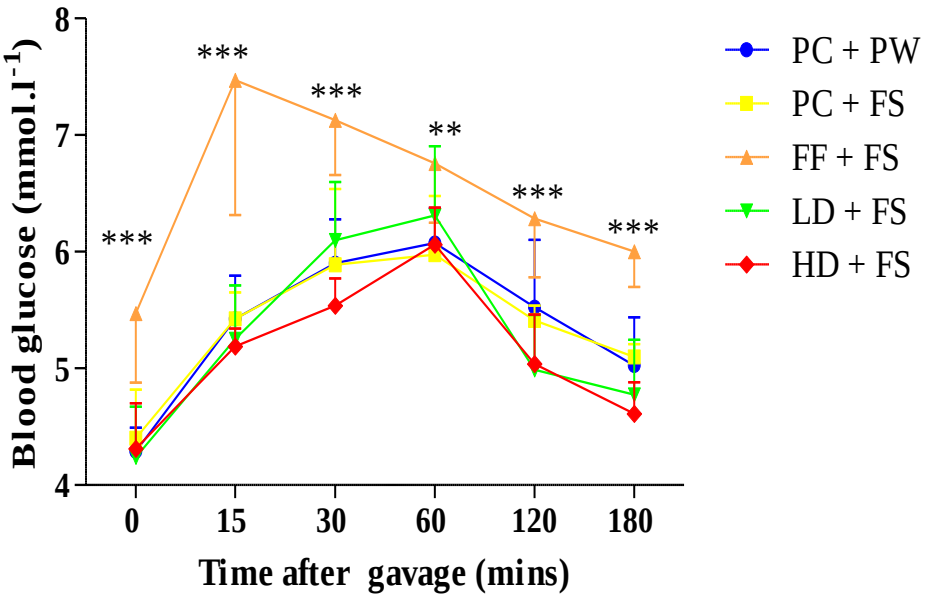

Figure 4.4A: Effect of aqueous *T. sericea* leaf extracts on the glucose tolerance of rats.

** P < 0.01; *** P < 0.0001. Rats given FF + FS had a significantly higher (P < 0.0001) blood glucose concentration compared to all the other treatment groups at all time intervals post gavage, except at 60 minutes post-gavage where they (rats given FF + FS) had significantly higher (P = 0.0006) blood glucose concentration compared to rats given PC + FS. The blood glucose concentration of rats given FF + FS significantly peaked (P < 0.0001) at 15 mins post-gavage while that of rats given other treatments significantly peaked (P < 0.0001) at 60 mins post gavage. PC + PW = plain gelatine cube + plain water; PC + FS = plain gelatine cube + 12% fructose solution; FF + FS = 100 mg.kg $^{-1}$ body mass per day fenofibrate + 12% fructose solution; LD + FS = low dose aqueous *T. sericea* leaf extract (100 mg.kg^{-1} body mass per day) + 12% fructose solution; HD + FS = high dose aqueous *T. sericea* leaf extract (400 mg.kg^{-1} body mass per day) + 12% fructose solution; Mins = minutes. Data presented as mean±SD; n = 8 per treatment.

The rats administered FF + FS had a significantly higher ($P < 0.0001$) basal blood glucose concentration and at 15, 30, 120, and 180 min post-gavage compared to rats given other treatments (Figure 4.4A). At 60 min post-gavage the rats that were given fructose solution and fenofibrate (FF + FS) had significantly ($P = 0.0006$) higher blood glucose concentration compared to their counterparts given plain cubes and fructose solution (PC + FS) (Figure 4.4A). While the blood glucose concentrations of rats given FF + FS returned to basal concentration 60 minutes post-gavage, those given LD + FS and HD + FS returned to basal 180 minutes post-gavage and those treated with PC + PW and PC + FS remained significantly higher ($P < 0.05$) compared to basal concentrations until 180 minutes post-gavage (Figure 4.4A).

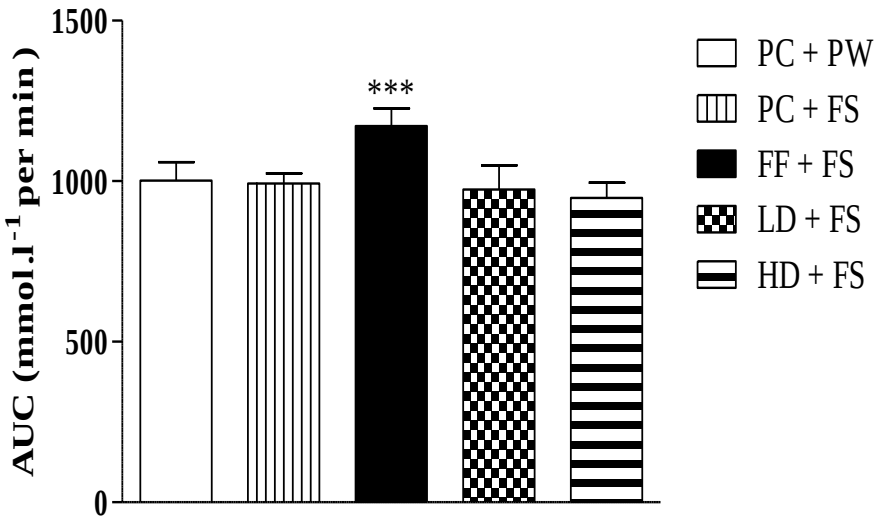

Figure 4.4B: Effect of aqueous *T. sericea* leaf extracts on total area under the curve of oral glucose tolerance of rats.

*** $P < 0.0001$. Rats treated with FF + FS had significantly ($P < 0.0001$) higher area under the curve of oral glucose tolerance test compared to that of rats from other treatment groups. PC + PW = plain gelatine cube + plain water; PC + FS = plain gelatine cube + 12% fructose solution; FF + FS = 100 mg.kg $^{-1}$ body mass per day fenofibrate + 12% fructose solution; LD + FS = low dose aqueous *T. sericea* leaf extract (100 mg.kg^{-1} body mass per day) + 12% fructose solution; HD + FS = high dose aqueous *T. sericea* leaf extract (400 mg.kg^{-1} body mass per day) + 12% fructose solution; AUC = Area under curve. Data presented as mean±SD; n = 8 per treatment.

The area under the curve of oral glucose tolerance test of rats admintered FF + FS was significantly ($P < 0.0001$) higher compared to the area under the curve of oral glucose tolerance test from rats given other treatment regimens, which (rats from other treatment groups) had similar ($P > 0.05$) area under the curve of oral glucose tolerance test (Figure 4.4B).

4.6 Effect of aqueous *T. sericea* leaf extracts plasma insulin concentration and HOMA index

Table 4.3 shows the plasma insulin concentrations and the HOMA-IR indexes of the rats after being given their respective treatments for 12 weeks.

Table 4.3 Effect of aqueous *T. sericea* leaf extracts on fasting blood glucose and plasma insulin concentration and HOMA-IR index

	PC + PW	PC + FS	FF + FS	LD + FS	HD + FS	Significance level
Glucose mmol.l^{-1}	4.14±0.36^{a}	4.01±0.44^{a}	5.14±0.54^{b}	4.06±0.35^{a}	3.95±0.36^{a}	***
Insulin µg.l^{-1}	5.65±2.80^{a}	5.32±2.28^{a}	5.93±2.21^{a}	5.40±1.73^{a}	5.21±1.50^{a}	ns
HOMA-IR	1.05±0.55^{a}	0.95±0.43^{a}	1.34±0.51^{a}	0.97±0.33^{a}	0.93±0.31^{a}	ns

n.s. = not significant, $P > 0.05$. ***$P < 0.0001$. ^{ab}Within row means with different superscripts are significantly different at $P \leq 0.05$. Rats given FF + FS had significantly higher ($P < 0.0001$) fasting glucose concentration compared to all rat treatment groups. The plasma insulin concentrations and HOMA-IR were statistically similar ($P > 0.05$) across for rats from all treatment groups. PC + PW = plain gelatine cube + plain water; PC + FS = plain gelatine cube + 12% fructose solution; FF + FS = 100 mg.kg $^{-1}$ body mass per day fenofibrate + 12% fructose solution; LD + FS = low dose aqueous *T. sericea* leaf extract (100 mg.kg^{-1} body mass per day) + 12% fructose solution; HD + FS = high dose aqueous *T. sericea* leaf extract (400 mg.kg^{-1} body mass per day) + 12% fructose solution; HOMA-IR = Homeostatic model of insulin resistance. Data presented as mean±SD; n = 8 per treatment.

While rats adminitered FF + FS had significantly elevated ($P < 0.0001$) fasting blood glucose concentration compared to that of rats from the rest of the treatments, the plasma insulin concentration and the HOMA-IR index were similar ($P > 0.05$) for the rats across the treatment groups (Table 4.3).

4.7 Effect of aqueous *T. sericea* leaf extracts on visceral organs

The effect of the treatments on the absolute and relative masses and lengths (where appropriate) of the GIT viscera is shown in Table 4.4A while the masses of the other visceral organs are shown in Table 4.4B.

Table 4.4A: Effect of aqueous *T. sericea* leaf extract on GIT visceral organ masses and lengths in male Wistar rats

Organ	PC + PW	PC + FS	FF + FS	LD + FS	HD + FS	Significance level
Stomach (g)	1.93±0.13^{a}	1.90±0.20^{a}	2.18±0.22^{a}	2.10±0.27^{a}	1.97±0.19^{a}	ns
%BM	0.54±0.03^{a}	0.54±0.06^{a}	0.64±0.04^{b}	0.57±0.05^{ab}	0.55±0.04^{a}	**
TLr (g.mm^{-1})	4.74±0.30^{a}	4.70±0.47^{a}	5.50±0.57^{b}	5.12±0.60^{ab}	4.84±0.44^{ab}	*
SI (mm)	1203±40.96^{a}	1243±102.4^{a}	1223±37.32^{a}	1172±89.17^{a}	1208±38.91^{a}	ns
SI (g)	7.41±0.71^{a}	7.27±0.48^{a}	8.38±1.03^{b}	7.60±0.60^{a}	7.31±0.41^{a}	*
%BM	2.07±0.14^{ab}	2.06±0.09^{ab}	2.47±0.15^{b}	2.08±0.08^{ab}	1.80±0.73^{a}	**
TLr (g.mm^{-1})	18.18±1.58^{a}	17.94±1.11^{a}	21.14±2.56^{b}	18.56±1.24^{a}	18.02±0.93^{a}	**
LI (mm)	248.10±20.34^{a}	268.80±14.33^{a}	261.30±14.58^{a}	254.40±10.16^{a}	260.00±18.52^{a}	ns
LI (g)	2.15±0.32^{a}	2.02±0.13^{a}	2.15±0.27^{a}	1.99±0.22^{a}	2.04±0.23^{a}	ns
%BM	0.60±0.07^{a}	0.57±0.04^{a}	0.64±0.09^{a}	0.54±0.06^{a}	0.57±0.06^{a}	ns
TLr (g.mm^{-1})	5.27±0.77^{a}	4.98±0.30^{a}	5.41±0.67^{a}	4.86±0.51^{a}	5.02±0.53^{a}	ns
Caecum (g)	1.48±0.24^{a}	1.38±0.25^{a}	1.22±0.18^{a}	1.33±0.24^{a}	1.29±0.24^{a}	ns

%BM	0.42±0.06[a]	0.39±0.05[a]	0.36±0.05[a]	0.36±0.06[a]	0.36±0.06[a]	ns
TLr (g.mm^{-1})	3.64±0.53[a]	3.40±0.62[a]	3.08±0.44[a]	1.33±0.24[b]	3.18±0.58[a]	***

n.s. = not significant, P > 0.05. * P ≤ 0.05; ** P < 0.01; ***P < 0.0001. [ab]Within row means with different superscripts are significantly different at P ≤ 0.05. Rats adminitered FF + FS had significantly (P < 0.01) heavier (relative to body mass) stomachs compared to rats treated with PC + PW, PC + FS and HD + FS. Rats administered FF + FS had significantly (P < 0.05) heavier (relative to tibia length) stomachs compared to rats treated with PC + PW and PC + FS. Rats given FF + FS had significantly (P < 0.05) heavier absolute and relative (to tibia length) small intestine masses compared to all rat treatment groups. Rats given FF + FS had significantly (P = 0.002) heavier (relative to body mass) small intestine masses compared to rats given LD + FS. Rats administered the LD + FS had significantly (P < 0.0001) lighter caeca (relative to tibia length) compared to other treatment groups. PC + PW = plain gelatine cube + plain water; PC + FS = plain gelatine cube + 12% fructose solution; FF + FS = 100 mg.kg $^{-1}$ body mass per day fenofibrate + 12% fructose solution; LD + FS = low dose aqueous *T. sericea* leaf extract (100 mg.kg^{-1} body mass per day) + 12% fructose solution; HD + FS = high dose aqueous *T. sericea* leaf extract (400 mg.kg^{-1} body mass per day) + 12% fructose solution; BM = Body mass; LI = Large intestine; SI = small intestine; TLr = Relative tibia length; VFP = visceral fat pad. Data presented as mean±SD; n = 8 per treatment.

Rats given FF + FS had significantly ($P < 0.01$) heavier stomachs relative to body mass compared to the stomach masses of rats given PC + PW, PC + FS and HD + FS (Table 4.4A). Relative to tibia length, rats administered FF + FS had significantly heavier stomachs ($P < 0.05$) compared to the stomach masses of rats treated with PC + PW and PC + FS (Table 4.4A). On the basis of absolute and relative to tibia length mass, rats administered FF + FS had significantly ($P < 0.05$) heavier small intestines compared to the small intestinal masses of rats administered other treatments (Table 4.4A). However relative to body mass the rats treated with FF + FS had significantly ($P = 0.002$) heavier small intestines compared to that of rats treated with LD + FS (Table 4.4A). Relative to tibia length, rats that were given FF + FS had significantly ($P < 0.05$) heavier caeca masses compared rats given other treatments (Table 4.4A). The large intestine absolute and relative (body mass and tibia length) masses and small intestine and large intestine lengths were similar ($P > 0.05$) across treatment groups (Table 4.4A).

Table 4.4B: Effect of aqueous *T. sericea* aqueous leaf extract on other visceral organs absolute and relative masses in male Wistar rats

Organs	PC + PW	PC + FS	FF + FS	LD + FS	HD + FS	Significance level
Heart (g)	1.24±0.11^{a}	1.24±0.12^{a}	1.28±0.14^{a}	1.23±0.16^{a}	1.16±0.06^{a}	ns
%BM	0.35±0.02^{ab}	0.35±0.03^{ab}	0.38±0.04^{b}	0.35±0.03^{ab}	0.32±0.01^{a}	*
TLr (g.mm^{-1})	3.04±0.24^{a}	3.06±0.28^{a}	3.23±0.36^{a}	3.12±0.40^{a}	2.87±0.16^{a}	ns
Liver (g)	10.31±1.12^{a}	10.56±1.11^{a}	16.36±2.01^{b}	10.68±1.11^{a}	10.42±0.88^{a}	***
%BM	2.88±0.20^{a}	3.00±0.28^{a}	4.83±0.32^{b}	2.92±0.18^{a}	2.92±0.12^{a}	***
TLr (g.mm^{-1})	25.30±2.70^{a}	26.10±3.01^{a}	41.25±5.01^{b}	23.86±2.66^{a}	25.66±1.94	***
Pancreas (g)	1.58±0.28^{a}	1.47±0.24^{a}	1.59±0.30^{a}	1.60±0.33^{a}	1.55±0.17^{a}	ns
%BM	0.44±0.07^{a}	0.41±0.06^{a}	0.47±0.10^{a}	0.44±0.08^{a}	0.43±0.04^{a}	ns
TLr (g.mm^{-1})	3.88±0.67^{a}	3.62±0.61^{a}	4.00±0.75^{a}	3.92±0.83^{a}	3.83±0.40^{a}	ns
VFP (g)	5.89±1.57^{a}	6.66±1.10^{a}	7.51±1.63^{a}	7.23±2.32^{a}	6.68±1.27^{a}	ns
%BM	1.64±0.37^{a}	1.88±0.26^{a}	2.21±0.35^{a}	1.96±0.54^{a}	1.87±0.31^{a}	ns

EFP (g)	4.86±0.93[a]	4.97±0.84[a]	4.38±1.12[a]	5.46±0.91[a]	4.90±0.68[a]	ns
%BM	1.36±0.22[a]	1.41±0.23[a]	1.28±0.25[a]	1.50±0.18[a]	1.37±0.18[a]	ns
Kidney (g)	2.53±0.24[a]	2.44±0.12[a]	2.90±0.26[b]	2.51±0.24[a]	2.50±0.16[a]	***
%BM	0.71±0.04[a]	0.69±0.03[a]	0.86±0.03[b]	0.68±0.04[a]	0.70±0.04[a]	***
TLr ($g.mm^{-1}$)	6.22±056[a]	6.01±0.36[a]	7.30±0.60[b]	6.13±0.59[a]	6.17±0.38[a]	***

n.s. = not significant, $P > 0.05$. *$P \leq 0.05$, ***$P < 0.0001$. [ab]Within row means with different superscripts are significantly different at $P \leq 0.05$. Rats administered FF + FS had significantly ($P = 0.0132$) heavier relative (to body mass) hearts masses compared to the mass of the hearts of rats administered HD + FS. Rats given FF + FS had significantly ($P < 0.0001$) heavier livers (absolute and relative to body mass and tibia length) compared to the liver masses of rats given the other treatments. Rats administered FF + FS had significantly ($P < 0.0001$) heavier kidneys (absolute and relative to body mass and tibia length) compared to the kidney masses from rats administered other treatments. PC + PW = plain gelatine cube + plain water; PC + FS = plain gelatine cube + 12% fructose solution; FF + FS = 100 $mg.kg^{-1}$ body mass per day fenofibrate + 12% fructose solution; LD + FS = low dose aqueous *T. sericea* leaf extract (100 $mg.kg^{-1}$ body mass per day) + 12% fructose solution; HD + FS = high dose aqueous *T. sericea* leaf extract (400 $mg.kg^{-1}$ body mass per day) + 12% fructose solution; BM = Body mass; EFP: Epididymal fat pad; TLr = Relative tibia length. Data presented as mean±SD; n = 8 per treatment.

While absolute heart masses across all treatment groups was similar ($P > 0.05$), relative to body mass rats administered FF + FS had significantly ($P = 0.0132$) heavier hearts compared to the heart masses of rats administered HD + FS (Table 4.4B). Relative to body mass and tibia length, rats that were administered FF + FS had significantly ($P < 0.0001$) heavier kidneys and livers, respectively, compared to the kidney and liver masses from rats received other treatments (Table 4.4B). The visceral fat pad, epididymal fat pad, pancreas and testicular masses were similar ($P > 0.05$) across all treatment groups (Table 4.4B).

Table 4.4C shows the villi height, crypt depth and ratio of the villi heights to crypt depths of the rats given the different treatments. The mean hepatocyte size and number of hepatocytes per 100µm linear field is shown in Table 4.4D.

Table 4.4C: Effect of aqueous *T. sericea* aqueous leaf extract on villi height, crypt depth and the villi height: crypt depth ratio in male Wistar rats

	PC + PW	PC + FS	FF + FS	LD + FS	HD + FS	Significance level
VH (µm)	51.83±2.42[a]	65.00±5.77[a]	66.12±7.95[a]	62.5±3.53[a]	58.5±9.19[a]	ns
CD (µm)	18.75±2.16[a]	19.50±1.00[a]	17.25±0.35[a]	17.75±4.95[a]	19.00±0.35[a]	ns
VH/CD ratio	2.76±1.12[a]	3.30±5.71[a]	3.83±0.54[a]	3.52±0.71[a]	3.08±26.26[a]	ns

n.s. = not significant, $P > 0.05$. [ab]Within row means with different superscripts are significantly different at $P \leq 0.05$. Villus height, crypt depth and the villus height: crypt depth ratios of the rats were similar ($P > 0.05$) across treatment groups. PC + PW = plain gelatine cube + plain water; PC + FS = plain gelatine cube + 12% fructose solution; FF + FS = 100 $mg.kg^{-1}$ body mass per day fenofibrate + 12% fructose solution; LD + FS = low dose aqueous *T. sericea* leaf extract (100 $mg.kg^{-1}$ body mass per day) + 12% fructose solution; HD + FS = high dose aqueous *T. sericea* leaf extract (400 $mg.kg^{-1}$ body mass per day) + 12% fructose solution; VH = Villus height; CD = Crypt depth. Data presented as mean±SD; n = 8 per treatment.

Table 4.4D: Effect of aqueous *T. sericea* leaf extract on hepatocyte size and density of male Wistar rats

Liver	PC + PW	PC + FS	FF + FS	LD + FS	HD + FS	Significance level
Hepatocyte size (µm)	9.37±0.90[a]	9.22±0.41[a]	9.81±0.54[a]	9.53±0.55[a]	9.67±0.84[a]	ns
Hepatocytes (cells per 100 µm)	10.53±0.90[a]	10.81±1.31[a]	10.41±0.48[a]	11.59±1.63[a]	12.13±1.46[a]	ns

n.s. = not significant, $P > 0.05$. [ab]Within row means with different superscripts are significantly different at $P \leq 0.05$. The number of intact hepatocytes in a linear field (100 µm) and hepatocyte size were statistically similar ($P > 0.05$) across all rat treatment groups. PC + PW = plain gelatine cube + plain water; PC + FS = plain gelatine cube + 12% fructose solution; FF + FS = 100 $mg.kg^{-1}$ body mass per day fenofibrate + 12% fructose solution; LD + FS = low dose aqueous *T. sericea* leaf extract (100 $mg.kg^{-1}$ body mass per day) + 12% fructose solution; HD + FS = high dose aqueous *T. sericea* leaf extract (400 $mg.kg^{-1}$ body mass per day) + 12% fructose solution. Data presented as mean±SD; n = 8 per treatment.

The villi height, crypt depth, villi height:crypt depth ratio, hepatocyte cell size and numbers of hepatocytes in a 100µm linear field were similar ($P > 0.05$) across all treatment groups (Tables 4.4C and 4.4D).

4.8 Effect of *T. sericea* aqueous leaf extracts on hepatic metabolic substrates

Figures 4.5A and 4.5B show liver glycogen (represented as glucose equivalents in the liver homogenate) and lipid content respectively, of the rats after being given their respective treatments for 12 weeks.

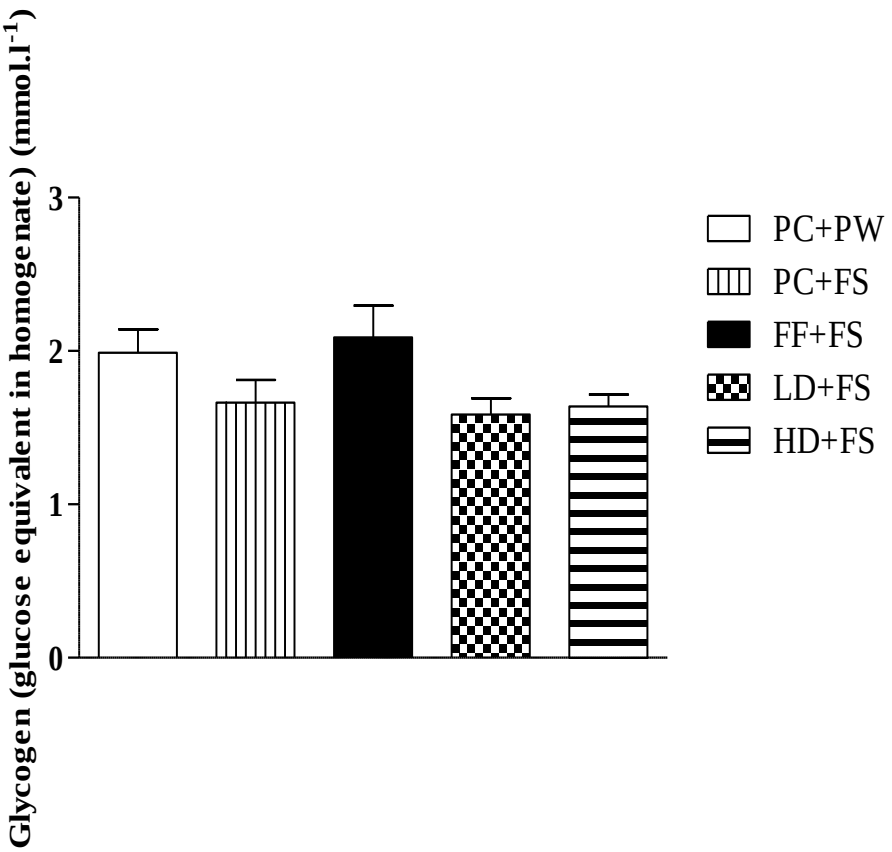

Figure 4.5A: Effect of aqueous *T. sericea* leaf extracts on the hepatic glycogen content of male Wistar rats.

Hepatic glycogen (represented as glucose equivalents in the liver homogenate) contents was statistically similar ($P > 0.05$) in the rats across the different treatment groups. PC + PW = plain gelatine cube + plain water; PC + FS = plain gelatine cube + 12% fructose solution; FF + FS = 100 mg.kg $^{-1}$ body mass per day fenofibrate + 12% fructose solution; LD + FS = low dose aqueous *T. sericea* leaf extract (100 mg.kg^{-1} body mass per day) + 12% fructose solution; HD + FS = high dose aqueous *T. sericea* leaf extract (400 mg.kg^{-1} body mass per day) + 12% fructose solution. Data presented as mean±SD; n = 8 per treatment.

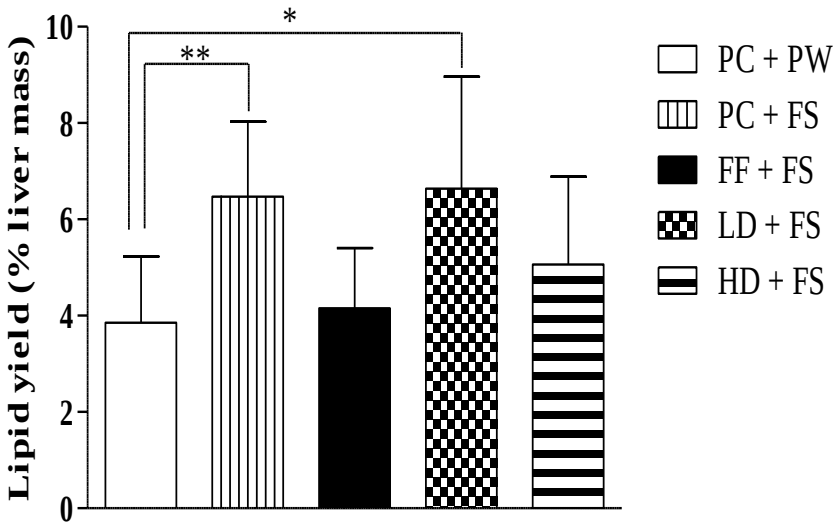

Figure 4.5B: Effect of aqueous *T. sericea* leaf extracts on the hepatic lipid content of male Wistar rats.

***** $P \leq 0.05$; ** $P < 0.01$. Rats given PC + FS and those given LD + FS had significantly ($P = 0.0032$; $P = 0.0114$, respectively) higher lipid yield (percentage liver mass) compared to rats given PC + PW (control). PC + PW = plain gelatine cube + plain water; PC + FS = plain gelatine cube + 12% fructose solution; FF + FS = 100 $mg.kg^{-1}$ body mass per day fenofibrate + 12% fructose solution; LD + FS = low dose aqueous *T. sericea* leaf extract (100 $mg.kg^{-1}$ body mass per day) + 12% fructose solution; HD + FS = high dose aqueous *T. sericea* leaf extract (400 $mg.kg^{-1}$ body mass per day) + 12% fructose solution. Data presented as mean±SD; n = 8 per treatment.

While the liver glycogen (represented as glucose equivalents in the liver homogenate) content of the rats across treatments were similar ($P > 0.05$; Figure 4.6B), rats that were given the plain gelatine cubes + 12% fructose solution (PC + FS) and their counterparts on a low dose of the aqueous *T. sericea* leaf extract + 12% fructose solution (LD + FS) had significantly higher ($P = 0.0032$; $P = 0.0114$, respectively) hepatic lipid content compared to rats that were given PC + PW (control) (Figure 4.6B).

4.9 Effect of aqueous *T. sericea* leaf extract on general health profile

Table 4.5 shows the effects on the markers of the general health of the rats following a 12 week administration of their respective treatments.

Table 4.5 The effect of aqueous *T. sericea* leaf extracts on serum markers of general health in male Wistar rats

Parameter	PC + PW	PC + FS	FF + FS	LD + FS	HD + FS	Significance level
ALT ($U.L^{-1}$)	64.13 ± 10.96^{a}	70.25 ± 18.61^{a}	80 ± 20.80^{a}	69.13 ± 10.60^{a}	70.25 ± 21.31^{a}	ns
ALP ($U.L^{-1}$)	112.6 ± 23.65^{a}	109 ± 19.12^{a}	347.40 ± 103.6^{b}	101.4 ± 19.26^{a}	102 ± 10.70^{a}	***
TBIL ($mg.dL^{-1}$)	0.4 ± 0.10^{a}	0.42 ± 0.14^{a}	0.46 ± 0.13^{a}	0.46 ± 0.12^{a}	0.38 ± 0.08^{a}	ns
Globulin ($g.dL^{-1}$)	2.01 ± 0.45^{a}	1.99 ± 0.64^{a}	1.71 ± 0.80^{a}	2.09 ± 0.55^{a}	1.76 ± 0.50^{a}	ns
Albumin ($g.dL^{-1}$)	3.41 ± 0.54^{a}	3.65 ± 0.62^{a}	4.05 ± 0.76^{a}	3.82 ± 0.44^{a}	3.82 ± 0.42^{a}	ns
Total protein ($g.dL^{-1}$)	5.45 ± 0.45^{a}	5.62 ± 0.54^{a}	5.77 ± 0.76^{a}	5.91 ± 0.40^{a}	5.57 ± 0.45^{a}	ns
BUN ($mg.dL^{-1}$)	19.88 ± 2.85^{a}	22.50 ± 2.78^{a}	23.88 ± 1.55^{a}	23.13 ± 2.36^{a}	23.25 ± 3.65^{a}	ns
Creatinine ($mg.dL^{-1}$)	0.40 ± 0.07^{a}	0.40 ± 0.12^{a}	0.38 ± 0.19^{a}	0.40 ± 0.13^{a}	0.45 ± 0.09^{a}	ns
Calcium ($mg.dL^{-1}$)	10.64 ± 0.94^{a}	10.88 ± 1.13^{a}	11.19 ± 2.04^{a}	11.06 ± 1.20^{a}	11.01 ± 0.82^{a}	ns
Phosphorus ($mg.dL^{-1}$)	5.46 ± 0.87^{a}	5.74 ± 0.51^{ab}	6.82 ± 1.16^{b}	6.22 ± 0.83^{ab}	5.54 ± 0.95^{ab}	*

Continued

Amylase ($U.L^{-1}$)	1349±271.9[a]	1522±232[a]	1588±306.4[a]	1495±114.2[a]	1424±51.98[a]	ns
Triglycerides ($mmol.l^{-1}$)	1.23±0.35[a]	1.53±0.37[a]	1.29±0.30[a]	1.38±0.27[a]	1.53±0.29[a]	ns
Cholesterol ($mg.dL^{-1}$)	69.75±10.74[a]	75.38±14.70[a]	75.38±31.30[a]	74.38±17.44[a]	74.25±10.47[a]	ns

* $P \leq 0.05$; *$P < 0.05$; ***$P < 0.0001$. [ab]Within row means with different superscripts are significantly different at $P < 0.05$. Rats administered FF + FS had significantly ($P < 0.0001$) higher serum ALP concentration compared to the serum ALP in rats given other treatments. Rats adminitered FF + FS had significantly ($P = 0.0346$) higher serum phosphate concentrations compared to that of rats given PC + PW. PC + PW = plain gelatine cube + plain water; PC + FS = plain gelatine cube + 12% fructose solution; FF + FS = 100 $mg.kg^{-1}$ body mass per day fenofibrate + 12% fructose solution; LD + FS = low dose aqueous *T. sericea* leaf extract (100 $mg.kg^{-1}$ body mass per day) + 12% fructose solution; HD + FS = high dose aqueous *T. sericea* leaf extract (400 $mg.kg^{-1}$ body mass per day) + 12% fructose solution; ALP = Alanine phosphatase ALT = Alanine aminotransferase; BUN = Blood urea nitrogen; TBIL = Total bilirubin. Data presented as mean±SD; n = 8 per treatment.

Rats that were given FF + FS had significantly ($P < 0.0001$) elevated serum ALP concentrations compared to the serum ALP concentrations of rats from other treatment groups (Table 4.5). The serum phosphate concentration from rats given FF + FS was significantly ($P = 0.0346$) elevated compared to that from rats given PC + PW (Table 4.5). Other serum markers of the general health profile were similar ($P > 0.05$) across the treatments (Table 4.5).

CHAPTER 5: DISCUSSION

The discussion focuses on the phytochemical composition of aqueous *T. sericea* leaf extracts, *in vitro* toxicity of the aqueous *T. sericea* leaf extract, effects of the leaf extracts on the growth performance, glucose tolerance and HOMA-IR index, GIT viscera and other visceral organs, hepatic metabolite storage and the general health profile of growing male Wistar rats allowed to drink 12% fructose solution *ad libitum*.

5.1 Phytochemical constituents

Bessong *et al.* (2004) reported the presence of triterpene saponin and tannins in organic *T. sericea* leaf extracts while Nkobole *et al.* (2011) noted organic stem bark *T. sericea* extracts to contain triterpene saponin, tannins and flavonoids. Although this study investigated, qualitatively, the phytochemical composition of the aqueous *T. sericea* extract, results of the current study are in agreement with the findings of Nkobole *et al.* (2011) and Bessong *et al.* (2004). Triterpene saponins, tannins and flavonoids, which were reported present in the aqueous leaf extract of the current study, are hypothesised to give *T. sericea* extracts antioxidant, lipolytic an antidiabetic properties (Subramaniam *et al.*, 2011; Singh *et al.*, 2008: Tapas *et al.*, 2008). The presence of the phytochemicals (triterpene saponins, tannins and flavonoids) in the *T. sericea* extracts points to potential antioxidant, lipolytic and hypoglycaemic properties.

5.2 *In vitro* toxicity on Brine shrimp nauplii

Our Animal Ethics Screening Committee (AESC) does not permit the use of animals in toxicity studies, therefore the Brine shrimp toxicity assay served as a proxy to screen the potential toxicity of the aqueous *T. sericea* extracts before use in rats. In the current study aqueous *T. sericea* aqueous leaf extracts were not toxic to brine shrimp nauplii with LC_{50} of (97.96 µg.ml^{-1} after 24 hours (Figure 4.1), which is above the 20 µg.ml^{-1} cytotoxic cut off point stated by Moshi and Mbwambo (2005). Moshi and Mbwambo (2005) reported that aqueous *T. sericea* root extracts were highly toxic to brine shrimp nauplii with LC_{50} of 5.4 µg.ml^{-1}. Findings of the current study on low toxicity to brine shrimp of aqueous *T. sericea* leaf extracts are corroborated in the findings of the general health profile of the rats, as will be discussed later in this dissertation. Futhermore, these findings suggest that aqueous *T. sericea* leaf extracts could be used in ethnomedicine with a reduced possibility of them causing toxicity.

5.3 Growth performance

5.3.1 Body mass

Body mass can be affected by hydration state, GIT content fill and visceral organ size (Owens *et al.*, 1995), thus making it an inaccurate indicator of growth performance. Empty carcass mass is a good indicator of growth (lean) performance (Owens *et al.*, 1995). In the current study the administration of PC + PW, PC + FS, LD + FS and LD + FS had no effect on empty carcass masses. However the administration of FS+FS caused a decrease in empty carcass mass of the rats. These findings suggest that while the administration of fenofibrate to rats fed 12% fructose solution caused a reduction in the empty carcass mass,

while administration of both a low and high dose aqueous *T. sericea* leaf extracts to rats fed a 12% fructose solution had no negative effect on the empty carcass mass.

5.3.2 Linear growth

Long bones grow in response to growth hormone in a dose-dependent manner (Rol De Lama *et al*., 2000), thus making them a better proxy for the estimation of growth performance (Sanchez and He, 2009; Han *et al.*, 1965; Butler *et al.*, 1956). The rats that were given FF + FS had significantly shorter tibiae compared to the tibiae lengths from rats given other treatment regimens (Table 4.2). Additionally they had lighter tibiae compared to their counterparts given LD + FS (Table 4.2). These findings suggest that the administration of 100 $mg.kg^{-1}$ body mass per day of fenofibrate to rats fed a 12% fructose solution might have adverse effects on lengths of tibia. However the similarity in tibiae and femora densities (as determined by computation and by radiography), from rats across treatments (Table 4.2 and Figure 4.3) suggest that the effects of fenofibrate on linear growth need further investigation.

5.4 Glucose tolerance, HOMA-IR and plasma insulin

Previous studies have reported that in rats, high fructose diets cause hyperglycemia, glucose intolerance, hypertriglyceridaemia, hypercholesterolaemia and insulin resistance (Atanasovska *et al.*, 2009; Huang *et al.*, 2004). The similarities in the fasting blood glucose concentrations (Table 4.3; Figure 4.4A), glucose handling after an oral glucose challenge

(Figure 4.4A and 4.4B), area under curve of oral glucose tolerance test, plasma insulin concentration, HOMA-IR (Table 4.3), fasting blood triglyceride and cholesterol (Table 4.5) of rats given the control (PC + PW), PC + FS, LD + FS and HD + FS indicates that a 12% fructose solution administered for 12 weeks to growing male Wistar rats did not cause hyperglycaemia, impaired glucose handling, hypertriglyceridaemia, hypercholesterolaemia and insulin resistance. The current study results are at variance with the reported hyperglycaemia, hypertriglyceridaemia and insulin resistance reported in adult Wistar rats after administration of a 10% fructose solution for 8 weeks (de Moura *et al.,* 2009). However, in a study on the effect of age on markers of metabolic syndrome in Wistar rats, Ghezzi *et al.* (2012) reported that at ages 2 to 6 months the rats had lower serum concentrations of glucose, triglycerides, total cholesterol, HDL and LDL compared to their 12-month old counterparts. At study termination the rats in the current study were 3.75-months old, thus were still in the age group where the rats were reported to have lower serum markers of metabolic syndrome (Ghezzi *et al.,* 2012). The failure to develop signs (glucose intolerance, hypertriglyceridaemia, insulin resistance and hypercholesterolaemia) of metabolic syndrome by rats in the current study after being administered with a 12% fructose solution for 12 weeks could be attributed to their age. de Moura *et al.* (2009) hypothesised that young Wistar rats may have protective mechanisms against fructose-induced metabolic syndrome however, they did not provide insight into the specific mechanisms that could be responsible. Further investigations are thus required in order to establish the underlying mechanisms.

It was observed in the current study that the administration of fenofibrate for 12 weeks at 100 $mg.kg^{-1}$ body mass per day to growing Wistar rats fed a 12% fructose solution resulted in decreased glycaemic control as evidenced by significantly higher fasting blood glucose

concentration (Table 4.3) and failure to effectively control blood glucose concentrations following an oral glucose load (Figure 4.4A and 4.4B) by rats that were given FF + FS. Liu *et al.* (2011) reported that the administration of 100 $mg.kg^{-1}$ body mass per day of fenofibrate for 12 weeks in monosodium glutamate-induced obese rats decreased glucose stimulated insulin release and worsened their ability to handle glucose. While fenofibrate is reported to cause inflammation and oxidative stress to the pancreas which results in decreased insulin secretion (Liu *et al.,* 2011) and decreased ability to handle glucose, the decreased glycaemic control and hyperglycaemia in the rats given FF + FS suggests that the administration of the fenofibrate may have affected pancreatic β-cell function.

The administration of 100 $mg.kg^{-1}$ fenofibrate for 9 days to 8-10 week old male Wistar rats with hypertriglyceridaemia reduced plasma triglyceride concentration (Ferreira *et al.,* 2008). In the current study, the administration of fenofibrate at a similar dose for 12 weeks to rats fed a 12% fructose had no effect on blood triglyceride concentration. A study by Furuhashi *et al.* (2002) showed that fenofibrate (30 $mg.kg^{-1}$) administration for 6 weeks to 12-week old Sprague-Dawley rats with insulin resistance improved peripheral insulin sensitivity; in the present study similarity in the HOMA-IR index of the rats given fenofibrate and that of their counterparts on other treatments suggested that fenofibrate had no effect on insulin sensitivity.

In the present study, the low and high dose of aqueous *T. sericea* leaf extracts had no effect on the fasting blood glucose, triglyceride, insulin and glucose handling of growing male Wistar rats. Tannins, saponins and flavonoids in *Terminalia* species extracts have been shown to reduce fasting blood glucose concentrations, improve glucose handling, insulin

sensitivity and β-cell function in streptozotocin-induced diabetic rats (Latha and Daisy 2010; Tapas *et al.,* 2008; Murali *et al.,* 2007; Nagappa *et al.,* 2003; Francis *et al.,* 2002). *In vitro* studies by Nkobole *et al.* (2011) and Tshikalange *et al.* (2008) attributed the antidiabetic properties of *T. sericea* to the bioactive constituients- tannins, saponins and flavanoids found in it. Findings of the current study, (fasting blood glucose, glucose tolerance test and HOMA-IR indexes) suggest that despite the presence of tannins, saponins and flavonoids in aqueous *T. sericea* leaf extracts, administration of both a low and high dose of the leaf extracts had no effect on fasting blood glucose and triglyceride concentration and insulin sensitivity by peripheral tissue.

5.5 Visceral organs

5.5.1 GIT visceral organs

Nutrients are mainly absorbed in the small intestine. An increased villi height:crypt depth ratio indicates increased absorptive ability (Metges, 2010). The development of the different parts of the GIT can be affected by dietary consumption (Metges, 2010). Fenofibrate administration to 3-month old PPARα null mice at dose 800 $mg.kg^{-1}$ in feed for 10 days has been shown to alter gene expression in the intestine (Valasek *et al.,* 2007) which translated to reduced lipid absorption (Uchida *et al.,* 2011; Valasek *et al.,* 2007) and increased height of villi in the small intestine (Uchida *et al.,* 2011; Valasek *et al.,* 2007). In the current study, the oral administration of fenofibrate (100 $mg.kg^{-1}$ body mass per day) for 12 weeks to rats fed a 12% fructose solution resulted in increased absolute and relative (to body mass and tibia length) small intestine mass (Table 4.4A) but had no effect on villus height and crypt depth (Table 4.4C). The effects of fenofibrate on the small intestine

could have been due to altered digestion and absorption resulting in an accumulation of nutrients the intestine, which might (nutrient accumulation), have resulted in stretching of the small intestine tissues and thus their increased masses (Tormo *et al*., 2004; Younes *et al.*, 2001). However, the mechanism by which fenofibrate increased the small intestine mass of growing rats given 12% fructose solution requires further investigation. The current study reports that whilst administration of fenofibrate (100 $mg.kg^{-1}$ body mass per day) resulted in increased relative (to body mass) stomach mass; administration of low dose (100 $mg.kg^{-1}$ body mass per day) of aqueous *T. sericea* extract resulted in increased relative (to tibia length) caeca mass in Wistar rats fed 12% fructose solution. However these finding could have been due to the differences in body mass and tibia length that the relative values were calculated from. Tannins have been reported to erode the GIT mucosal layer (Chung *et al.*, 1998) and thus interfere with the digestive and absorptive function of the GIT. In the current study, the administration of aqueous *T. sericea* leaf extracts that contained tannins for 12 weeks had no negative effect on growth performance (body mass and linear), suggesting that GIT's digestive and absorptive function was not compromised hence nutrients were availed to support growth.

5.5.2 Other viscera

High fructose diets are known to have the potential to cause cardiovascular and renal system complications, such as microvascular and muscular tissue hypertrophy (Dachani *et al.*, 2012; Gersch *et al.*, 2007; Sánchez-Lozada *et al.*, 2007). Furthermore, they are known to increase liver mass and visceral fat pad mass (London and Castonguay, 2011; Huang *et al.*, 2004). In the current study following the administration of PC + FS to rats for 12

weeks, no changes in absolute and relative masses of the heart, kidney, visceral fat pad, epididymal fat pad, liver and pancreas were observed in growing Wistar rats. However, following the administration of FF + FS for 12 weeks, results of the current study point to increased heart relative (to body mass) masses in the growing Wistar rats (Table 4.4B) which are suggestive of increased cardiac tissue. Similar results were reported by Duhaney *et al.* (2007) where fenofibrate administration was found to result in increased ventricular hypertrophy in wild-type FVB mice. Thus, findings from the current suggest that the oral administration of fenofibrate might have adverse effect on the heart mass of Wistar rats given a standard rat chow but given a 12% fructose solution. However, since there were no statistically significant differences absolute masses of the hearts from the rats across treatments, this finding requires further investigation.

Fenofibrate has been shown to be reno-protective in metabolic syndrome models using adult mice and rats (Tanaka *et al.*, 2011; Park *et al.*, 2006). Results of the current study revealed that administration of fenofibrate ($100mg.kg^{-1}$ body mass per day) for 12 weeks to growing Wistar rats fed a 12% fructose solution, caused a significant ($P < 0.05$) increase in absolute and relative (to body mass and tibia length), in the masses of their kidneys (Table 4.4B). Kidney hypertrophy could be a result of microvascular infiltration of the kidney tissue which has potential to cause kidney disease, thus results of the current study seem to contradict the reported reno-protecitve effects of fenofibrate (Tanaka *et al.*, 2011; Park *et al.*, 2006).

Price *et al.* (1986) reported that fenofibrate can result in hepatomegally within 3 days of administration in rats. Hong *et al*. (2007) reported that the administration of fenofibrate

at100mg.kg^{-1} body mass per day for 6 weeks to adult male Sprague-Dawley rats resulted in hepatomegally. Clinical studies have demonstrated that fenofibrate causes inflammation of the liver which in turn leads to hepatomegally (Tolman, 2000). In the current study, the administration of fenofibrate (100mg.kg^{-1} body mass per day) for 12 weeks to growing male Wistar fed a 12% fructose solution caused a significant increase in absolute and relative (to body mass and tibia length) liver mass which might be indicative of hepatomegally (Table 4.4B), but without affecting hepatocyte size and the number of hepatocytes in a linear field (Table 4.4D). Results the current study are in agreement with the finding reported by Gasa (2012), Hong *et al.* (2007), Harano *et al.* (2006) and Price *et al.* (1986) also reported that fenofibrate causes hepatomegally.

The tannins, saponins and flavonoids of *Terminalia arjuna* and *Terminalia chebula* have been shown to be protective to the heart, kidney, liver and pancreas in alloxan-induced diabetic rats, adult male Wistar, NZW albino and Swiss albino rats (Muhammad *et al.*, 2012; Raghavan and Kumari, 2008; Sinha *et al.*, 2008; Manna *et al.*, 2006; Gauthaman *et al.*, 2005; Suchalatha and Shyamala, 2004; Gauthaman *et al.*, 2001; Dwivedi and Agarwal, 1994). In the current study following administration of aqueous *T. sericea* leaf extracts at low (100 mg.kg^{-1} body mass per day) and high (400 mg.kg^{-1} body mass per day) doses for 12 weeks to growing Wistar rats fed a 12% fructose solution, no changes in absolute and relative masses of the heart, kidney, visceral fat pad, epididymal fat pad, liver and pancreas were observed. These finding suggests that aqueous *T. sericea* leaf extracts might have no adverse effects on these viscera.

5.6 Liver metabolic substrate storage and markers of general health

The liver has several functions including: metabolite storage, bile production, protein synthesis, and inactivation of chemical messengers and detoxification (Govind, 2011; Salie *et al.,* 1991). High fructose diets are known to cause liver diseases by altering the metabolism of carbohydrates and lipids that leads to derangement of homeostasis (Rutledge and Adeli 2007; Thirunavukkarasu *et al.,* 2004). Conventional drugs and ethnomedicines may contain contaminants and toxins which are processed and detoxified in the liver and may have the potential to negatively influence liver function (Basha *et al.,* 2012; Hajdu *et al.*, 2009; Tasduq *et al.*, 2006; Manna *et al.,* 2006). It is therefore important to measure surrogate markers of liver function when ethnomedicines are used.

5.6.1 Liver glycogen content

Feeding a high-fructose diet to Wistar rats has been reported to reduce glycogen synthesis in the liver through reduced sensitivity to insulin by hepatocytes, which translates to decreased glucose uptake by the cells (Bezerra *et al.,* 2001). Decreased glucose uptake by hepatocytes results in reduced glycogen synthesis (Kumar *et al.,* 2006). Yadav *et al.* (2007) reported that administering 21% fructose solution for 8 weeks to 8-week old Wistar rats significantly increased their liver glycogen content. In the current study following administration of respective treatments for 12 weeks, no differences were observed in the liver glycogen content of rats given to the control (PC + PW), PC + FS and those on the low (LD + FS) and high (HD + FS) dose aqueous *T. sericea* leaf extract and those given fenofibrate (FF + FS). These findings suggest that the 12% fructose solution, fenofibrate

(100 mg.kg^{-1} body mass per day), and low (100 mg.kg^{-1} body mass per day) and high (400 mg.kg^{-1} body mass per day) aqueous *T. sericea* extracts did not affect fasting liver glycogen content of Wistar rat. Fasting is known to cause liver glycogen depletion (Gutman and Shafrir, 1964). The similarity in the liver glycogen content from the rats across treatments could have been due to the depletion of the liver glycogen stores, since the rats were fasted overnight prior to euthanasia. Future studies should consider investigating glycogen stores of rats in the fed state.

5.6.2 Liver lipid content

High fructose diets are known to cause increased lipid synthesis and storage by the liver, viscera and muscles (Le *et al.*, 2009; Bergheim *et al.*, 2008; Le *et al.*, 2006). In the current study administration of PC + FS and LD + FS for 12 weeks resulted in increased hepatic lipid content in growing Wistar rats (Figure 4.5B). This finding suggests that the 12% fructose solution induced increased lipid storage by the liver which can potentially cause fatty liver disease. Fatty liver disease is a liver-based manifestation of the development of metabolic syndrome (Song *et al.*, 2013; de Moura *et al.*, 2009). Fenofibrate is a lipid lowering drug (Staels *et al.*, 1998) and has been shown to reduce hepatic lipid synthesis and storage in 10-week old Wistar rats (Ferreira *et al.*, 2008). The current study reports similar results, that fenofibrate inhibited high fructose diet induced lipid accumulation in the liver (Figure 4.5B). *In vitro* studies have shown that triterpene saponin extracted from *T. sericea* root has lipolysis promoting properties (Mochizuki and Hasegawa 2006). In the current study the high dose of aqueous *T. sericea* leaf extract inhibited the fructose diet induced accumulation of lipids in the liver (Figure 4.5B) suggesting that at a high dose, the

aqueous *T. sericea* leaf extracts might prevent accumulation of lipids in the liver, thus exhibiting protective potential against diet induced fatty liver disease.

5.6.3 Markers of liver function

Alanine aminotransferase (ALT) is a surrogate biomarker for intra-hepatic damage (Thulin *et al.,* 2008). Alkaline phosphatase (ALP) is a biomarker that indicates post-hepatic liver damage or increased bone formation if plasma concentrations are elevated (Pratt and Kaplan, 2000; Reichling and Kaplan, 1988). Bilirubin is processed to a soluble state by the liver and then excreted in urine and faeces. Elevated concentrations of bilirubin can be indicative of red blood cell hemolysis or liver damage (Pratt and Kaplan, 2000). Albumin and globulin are major serum proteins that are synthesized by the liver. Decreased albumin and globulin serum concentrations are indicative of end-stage liver disease amongst other diseases (Oettl *et al.,* 2013).

In rats, high fructose diets have been reported to induce non-alcoholic fatty liver disease (NAFLD). The latter (NAFLD) has been implicated as one of the causes of increased serum ALT transaminase (de Catro *et al.,* 2013). Interestingly, while other research has noted that high fructose diets led to increased fat deposition in the liver and subsequent NAFLD (Castro *et al.,* 2011; Kanuri *et al.,* 2011), it could be inferred from this current study that although the feeding of a 12% fructose solution to growing Wistar rats for 12 weeks caused “fatty” livers, it did not result in increased serum ALT and ALP concentrations (Table 4.5).

The administration of fenofibrate has been shown to cause an increase in serum ALT and ALP concentration (Hajdu *et al.,* 2009; Ho *et al.,* 2004) due to hepatocellular damage (Tolman, 2000). Interestingly, Cindoruk *et al*. (2007) reported that administration of fenofibrate reduced serum total bilirubin, ALT and ALP in adult male Wistar rats with obstructive liver disease. Results of the current study showed that the administration of fenofibrate (100 $mg.kg^{-1}$ body mass per day) for 12 weeks to growing Wistar rats fed a 12% fructose solution caused increased serum ALP concentration (Table 4.5), thus suggesting possible hepatic damage by fenofibrate. However, that fact that other serum markers of liver function (ALT, total bilirubin, albumin and globulin) were not affected suggest that the elevated ALP could have been from bone or intestinal cells (Wolf, 1999), since high bone turnover in growing animals is associated with increased serum concentrations of ALP (Pratt and Kaplan, 2000; Wolf, 1999; Reichling and Kaplan, 1988). Syversen *et al.* (2009) reported that PPARα increases osteoblast activity which in turn results in elevated blood ALP. An isoenzyme of ALP is also found in intestinal cells (Wolf, 1999), thus the increased blood ALP in rats given FF + FS could have been due to the increased intestinal tissue which was noted in the current study. The specific isoenzyme of ALP that was increased was not identified in the current study. Further investigations are required to identify the possible source (liver, bone and liver) of the increased blood ALP concentration following fenofibrate administration to growing rats.

Terminalia species (*T. chebula*, *T. arjuna* and *T. brownii*) have hepatoprotective properties and are used in ethnomedicine to treat liver diseases (Basha *et al.,* 2012; Manna *et al.,* 2006; Tasduq *et al.,* 2006). Manna *et al.* (2006) showed that aqueous extracts of *T. arjuna* stem bark lowered serum ALP in Swiss-albino rats with carbon tetrachloride induced liver injury. In the current study the low and high dose of aqueous *T. sericea* leaf extracts had no

effect on serum albumin, ALT and ALP concentrations of growing Wistar rats fed 12% fructose solution, which suggests that the oral administration of aqueous *T. sericea* leaf extracts did not cause liver damage further confirming the *in vitro* findings using the brine shrimp nauplii.

5.6.4 Markers of kidney function

Creatinine and blood urea nitrogen (BUN) are excreted by the kidney (Chen *et al.,* 2011b). Elevated serum concentrations of creatinine and BUN indicate renal complications or increased muscle catabolism (Chen *et al.,* 2011b; Cavalcanti *et al.,* 2006). In adult Sprague-Dawley rats high fructose (60% w/w) feeds consumed over a 6-week period have been demonstrated to cause kidney damage and decrease their ability to filter, thus increasing blood creatinine and BUN concentrations (Gersch *et al.,* 2007). In the current study the administration if 12% fructose solution for 12 weeks had no effect on serum creatinine and BUN, suggesting that the renal function of the rats' kidneys (across treatments) was not compromised.

Chronic use of fenofibrate is known to increase serum creatinine and BUN in humans (Chen *et al.,* 2011b). However the mechanisms by which it causes this are still not fully understood. Researchers have suggested that fenofibrate possibly reduces glomerular filtration (Chen *et al.,* 2011b). In the current study there was no elevation of serum creatinine and BUN concentrations from the growing Wistar rats following administration of fenofibrate (100 $mg.kg^{-1}$ body mass per day) for 12 weeks, suggesting that fenofibrate did not reduce glomerular filtration.

Terminalia arjuna aqueous stem bark extract has been shown to protect the kidneys against oxidative stress induced damage (Manna *et al.,* 2006) whilst *Terminalia bellerica* organic fruit extract administration to adult Streptozotocin diabetic rat for 60 days, reduced serum creatinine and urea (Latha and Daisy, 2010). In the current study, administration of aqueous *T. sericea* leaf extracts for 12 weeks did not affect serum creatinine and BUN in growing Wistar rats suggesting that *T. sericea* aqueous leaf extracts had no adverse effects on kidney function.

5.6.5 Effect on serum calcium and phosphorus concentrations

Calcium and phosphorus can be absorbed from the GIT or kidneys and are involved in bone formation and mineralization**.** Their serum concentration are mainly regulated via parathyroid and calcitonin hormone but are also be influenced by diet (de Paula and Rosen, 2010). Calcium and phosphorus concentrations can thus be indicative of bone activity (resorption and formation), GIT mineral absorption and kidney mineral absorption and secretion. Fructose has been shown to cause kidney damage that can result in increased calcium and phosphorus excretion (Taylor and Curhan, 2008; Levin *et al.,* 2007). In the current study the administration of PC + FS did not result in increased excretion of the two minerals suggesting that there was no damage to the nephrons. However results of the current study show that the administration of fenofibrate (100 $mg.kg^{-1}$ body mass per day) for 12 weeks to growing Wistar rats fed a 12% fructose solution resulted in increased serum phosphorus concentration (Table 4.5). This could have been due to increased absorption in GIT and or reabsorption in the kidney. However, further research needs to be

conducted to investigate mechanisms by which fenofibrate increased serum phosphorus. Tannins are known to have potential to reduce the absorption of minerals (calcium and phosphorus) and proteins from the GIT (Muhammad and Oloyede, 2004). Although in the current study it was qualitatively established that the aqueous *T. sericea* leaf extracts contained tannins, the similarity in the serum calcium and phosphorus concentration in rats which were given both a low and high dose of the aqueous *T. sericea* leaf extracts for 12 weeks suggests that the tannins in the extract did not negatively affect their absorption from the GIT.

5.6.6 Effect on serum cholesterol

Elevated serum cholesterol levels are associated with various systemic diseases such as metabolic syndrome, diabetes mellitus and cardiovascular diseases (Neuschwander-Tetri and Wang, 2013). While the oral administration of a 21% fructose solution in 8-week old Wistar rats for 8 weeks caused elevated serum cholesterol concentrations (Yadav *et al.,* 2007), in the current study the oral administration of 12% fructose solution for 12 weeks did not cause hypercholesterolaemia in growing Wistar rats. Fenofibrate is known to reduce serum cholesterol by inhibiting lipid absorption in the gut and promoting lipid oxidation (Valasek *et al.,* 2007; Jeong *et al.,* 2004b). In the current study, no changes in serum cholesterol concentration were observed following the oral fenofibrate administration for 12 weeks in growing Wistar rats given a 12% fructose solution. Saponins and tannins from *Terminalia arjuna* are known to lower cholesterol synthesis by inhibiting lipid absorption and synthesis in the gut and liver, and by increasing high density lipoprotein synthesis (Ram *et al.,* 1997; Tiwari *et al.,* 1990). *T. sericea* aqueous leaf

extracts that were administered to growing Wistar rats for 12 weeks contained saponins and tannins; however they did not affect serum cholesterol concentrations. It should be noted that the effects of the treatments on serum cholesterol could have been affected by the fact that the rats were not on a high fat diet.

5.6.7 Effect on serum amylase activity

Amylases which catalyse the hydrolysis of starch and glycogen to glucose are found in saliva and exocrine pancreatic secretions. Their serum concentrations can be used as markers of inflammation of the salivary gland or pancreas (Huang *et al.*, 2004; Pieper-Bigelow *et al.*, 1990). Amylases are targets for diabetes medications: inhibition of amylases reduces glucose absorption and thus reducing hyperglycaemia (Hasenah *et al.*, 2006). Huang *et al.* (2004) observed that in 7-week old Sprague-Dawley rats given a high-fructose (60%) diet for 8 weeks did not affect serum amylase concentrations. Similarly results of the current study show that oral administration of 12% fructose solution for 12 weeks had no effect on serum amylase concentration in growing Wistar rats. This suggests that 12% fructose solution did not cause inflammation to the salivary glands and pancreas. Furthermore administration of fenofibrate had no effect on the amylase concentration in growing Wistar rats, suggesting that oral administration of fenofibrate did not cause pathology in salivary glands and or that of the pancreas. While findings from the *in vitro* research done by Nkobole *et al.* (2011) showed that *T. sericea* stem bark tannins, saponins and flavonoids inhibit amylase, in the current study, *in vivo*, aqueous *T. sericea* leaf extracts had no effect on amylase serum concentration in growing Wistar rats. The effects of *T. sericea* on amylases and carbohydrate absorption *in vivo* need to be further investigated.

CHAPTER 6: CONCLUSION AND RECOMENDATIONS

6.0 Conclusion and recommendations

The study evaluated the phytochemical composition of aqueous *T. sericea* leaf extracts, toxicity of the aqueous *T. sericea* leaf extract *in vitro*, effects of the administration of the aqueous leaf extracts on the growth performance, glucose tolerance and HOMA-IR index, GIT viscera and other visceral organs, hepatic metabolite storage and the general health profile of growing male Wistar rats fed 12% fructose solution. Using qualitative methods, *T. sericea* leaf extracts were found to contain pharmacologically active phytochemicals and to be non-toxic to Brine shrimp nauplii. Future studies should quantify the phytochemical constituents of the aqueous *T. sericea* leaf extracts and investigate the toxicity of aqueous *T. sericea* leaf extracts *in vivo*.

Findings of the current study showed that while the oral administration of a 12% fructose solution for 12 weeks to growing male Wistar rats resulted in the development of a "fatty liver", one of the markers of metabolic syndrome, it did not cause the development of hyperglycaemia, hypertriglyceridaemia, hypercholesterolaemia, insulin resistance and central obesity, which are the other markers of metabolic syndrome. The administration fenofibrate and high dose of *T. sericea* extracts protected against the development of a high fructose diet-induced fatty liver. The mechanisms of how the high dose of *T. sericea* extracts protected against development of a "fatty liver" need to be further investigated in non-alcoholic fatty liver disease and obesity models. The protective effects of aqueous *T. sericea* leaf extracts can be further exploited clinically in the prevention and possibly treatment of patients with fatty liver disease. The oral administration of a low dose (100 $mg.kg^{-1}$ body mass per day) and high dose (400 $mg.kg^{-1}$ body mass per day) of aqueous *T.*

sericea leaf extracts did not negatively affect growth performance (body mass, empty carcass mass and indexes of long bone growth).

While the administration of aqueous *T. sericea* leaf extracts did not improve glucose handling following an oral glucose challenge, the extracts had no adverse effect on the rats' glucose tolerance, plasma insulin concentration, visceral organ morphometry and on markers of general health profile of the rats. These observations suggest that aqueous *T. sericea* leaf extracts could be used in ethnomedicine without an increased risk of compromising the glycaemic control, growth performance, GIT and other viscera's morphometry and general health. The oral administration of fenofibrate (100 $mg.kg^{-1}$ body mass per day) to growing Wistar rats fed a 12% fructose solution for 12 weeks reduced empty carcass, reduced linear growth of the tibia, caused hyperglycaemia, possible liver injury and increased mass of visceral organs (small intestine, liver and kidney) in growing Wistar rats. The adverse effects of the chronic administration of fenofibrate on tibia bone growth, blood glucose homeostasis and liver function enzymes need to be further investigated at a cellular and molecular level.

6.1 Limitations

In the current study administration of 12% fructose solution *ad libitum* resulted in increased liver lipid ("fatty liver") however, no other signs of metabolic dysfunction in growing Wistar rats were observed possibly due to it not providing enough excess calories. Therefore future studies should consider using fructose solution at a higher concentration and possibly supplementing the standard rat chow with lard, to increase calorie and fat intake. The present study used male rats only, future studies should consider investigating

the effects of orally administered aqueous *T. sericea* extracts and fenofibrate in male and female rats fed normal rat chow and plain water as this will allow for an investigation of the effects of the extracts on normal rats of different rat genders. They (future studies) should also consider investigating and comparing the effects of *T. sericea* organic extracts to aqueous extracts. Additionally, future studies should also investigate the effects of oral administration of aqueous *T. sericea* leaf extracts on nutrient absorption from the GIT. While the current study did not employ molecular techniques to elucidate some of the mechanisms that may have been at play, it is recommended that future studies include molecular techniques in order to get measurements gene and protein expression level as this would helps better explain the mechanisms behind the findings of the study. Other technical limitations include that only qualitative and not quantitative phytochemical analyses were performed on *T. sericea* leaf extracts and that subjective assessment of radiographs was done to analyse bone density of the rats. Future studies should perform quantitative analyses on *T. sericea* leaf extracts and also do densitometry to analyse bone density of the rats. While it would have been interesting to investigate the effects of aqueous *T. sericea* leaf extracts alone, the current study was more focused on the effects of fructose induced changes as opposed to general effects of aqueous *T. sericea* leaf extracts which would have required a different experimental design.

CHAPTER 7: REFERENCES

Aganga, AA, Adogla-Bessa T, Omphile UJ and Tshireletso K (2000). Significance of browses in the nutrition of Tswana goats. *Archivos de Zootecnia* 49: 469-480

Ahmed SM, Swamy BMV, Dhanapal PGR and Chandrashekara VM (2005). Anti-diabetic activity of *Terminalia catappa* linn. leaf extracts in alloxan-induced diabetic rats. *Iranian Journal of Pharmacology and Therapeutics* 41: 36-39

Al-Isa A (2013). Prevalence of metabolic syndrome(MetS) among male Kuwaiti adolescentsaged 10 - 19 years. *Health* 5: 938-942

Alberti KGMM, Zimmet P and Shaw J (2006). Metabolic syndrome-a new world-wide definition. A consensus statement from the international diabetes federation. *Diabetic Medicine* 23: 469-480

Al-Sarihin K, Althwabia I, Khaled MB and Haddad F (2013). Prevalence of peripheral neuropathy among patients with diabetes mellitus at King Hussein Hospital, Amman, Jordan. *Rawal Medical Journal* 38: 92-96

Anderson DMW and Bell PC (1974). The composition and properties of gum exudates from *Terminalia sericea* and *T. superba*. *Phytochemistry* 13: 1871-1874

Arnold HJ and Gulumian M (1984). Pharmacopoeia of traditional medicine in Venda. *Journal of Ethnopharmacology* 12: 35-74

Atanasovska E, Jakovski K, Kostova E, Petlichkovski A, Dimitrovski C, Bitovska I, Kikerkov I, Petrovski O and Labachevski N (2009). Effects of Rosiglitazone on metabolic parameters and adiponectin levels in fructose-fed rats. *Macedonian Journal of Medical Sciences* 2: 22-29

Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC and Atangbayila TO (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in South western Nigeria. *Tropical Journal of Pharmaceutical Research* 7: 1019-1024

Ayyanar M, Sankarasivaraman K and Ignacimuthu S (2008). Traditional herbal medicines used for the treatment of diabetes among two major tribal groups in South Tamil Nadu, India. *Ethnobotanical Leaflets* 12: 276-280

Basha NS, Ogbaghebriel A, YemaneK and Zenebe M (2012). Isolation and screening of endophytic fungi from Eritrean traditional medicinal plant *Terminalia brownii* leaves for antimicrobial activity. *International Journal of Green Pharmacy* 6: 40-44

Basciano H, Federico L and Adeli K (2005). Fructose, insulin resistance, and metabolic dyslipidemia. *Nutrition and Metabolism* 5: 1-14

Beilby J (2004). Definition of metabolic syndrome: report of the national heart, lung, and blood institute/American heart association conference on scientific issues related to definition. *Circulation* 109: 433-438

Bell RC, Carlson JC, Storr KC, Herbert K and Sivak J (2000). High-fructose feeding of streptozotocin-diabetic rats is associated withincreased cataract formation and increased oxidative stress in the kidney. *British Journal of Nutrition* 84: 575-582

Bergheim I, Weber S, Vos M, Kramer S, Kaserouni VVS, McClain CJ and Bischoff SC (2008). Antibiotics protect against fructose-induced hepatic lipid accumulation in mice: Role of endotoxin. *Journal of Hepatology* 48: 983-9

Bertram CE and Hanson MA (2001). Animal models and programming of the metabolic syndrome. *British Medical Bulletin* 60: 103-121

Bessong PO, Obi CL, Igumbor E, Andreola M and Litvak S (2004). *In vitro* activity of three selected South African medicinal plants against human immunodeficiency virus type 1 reverse transcriptase. *African Journal of Biotechnology* 3: 555-559

Bezerra RMN, Ueno M, Silva MS, Tavares DQ, Carvalho CRO, Saad MJA and Gontijo JAR (2001). A high-fructose diet induces insulin resistance but not blood

pressure changes in normotensive rats. *Brazilian Journal of Medical and Biological Research* 34: 1155-1160

Bligh EG and Dyer WJ (1959). A rapid method of total lipid extraction and purification. *Journal of Biochemistry and Physiology* 37: 911-917

Bowyer P, Clarke BR, Lunness P, Daniels MJ and Osbourn AE (1995). Host range of a plant pathogenic fungus determined by a saponin detoxifying enzyme. *Science* 267: 371-373

Buettner R, Parhofer KG, Woenckhaus M, Wrede CE, Kunz-Schughart LA, Schölmerich J and Bollheimer LC (2006). Defining high-fat-diet rat models: metabolic and molecular effects of different fat types. *Journal of Molecular Endocrinology* 36: 485-501

Busserolles J, Gueux E, Rock E, Demigne C, Mazur A and Rayssiguier Y (2003). Oligofructose protects against the hypertriglyceridemic and pro-oxidative effects of a high fructose diet in rats. *Journal of Nutrition* 133: 1903-1908

Bussmann RW, Malca G, Glenn A, Sharon D, Nilsen B, Parris B, Dubose D, Ruiz D, Saleda J, Martinez M, Carillo L, Walker K, Kuhlman A and Townesmith A (2011). Toxicity of medicinal plants used in traditional medicine in Northern Peru. *Journal of Ethnopharmacology* 137: 121-140

Butler OD, Warwick BL and Cartwright TC (1956). Slaughter and carcass characteristics of short-fed yearling Hereford and Brahman x Hereford steers. *Journal of Animal Science* 15: 93-96

Castro GSF, Cardoso JFR, Vannucchi H, Zucoloto S and Jordão AA (2011). Fructose and NAFLD: metabolic implications and models of induction in rats. *Acta Cirúrgica Brasileira* 26: 45-50

Cavalcanti AM, Baggio CH, Freitas CS, Rieck L, de Sousa RS, Da Silva-Santos JE, Mesia-Vela S and Marques MCA (2006). Safety and antiulcer efficacy studies of *Achilleamillefolium* L. after chronic treatment in Wistar rats. *Journal of Ethnopharmacology* 107: 277-284

Chaudhari M and Mengi S (2006). Evaluation of phytoconstituents of *Terminalia arjuna* for wound healing activity in rats. *Phytotherapy Research* 20: 799-805

Chen L, Magliano DJ and Zimmet PZ (2011)a. The worldwide epidemiology of type 2 diabetes mellitus-present and future perspectives. *Endocrinology* 8: 228-236

Chen Y, Hsu C, Huang W, Chen C, Lee P, Chang T, Chou K, Chung H and Fang H. (2011)b. Fenofibrate reversibly increases serum creatinine level in chronic kidney disease patients by reducing glomerular filtration rate. *Acta Nephrologica* 25: 1-4

Cheung N Wang JJ, Klein R, Couper DJ, Sharrett AR, and Wong TY (2007). Diabetic retinopathy and the risk of coronary heart disease. *Diabetes Care* 30: 1742-1746

Chivandi E, Davidson BC and Erlwanger KH (2013). Proximate, mineral, fibre, phytate-phosphate, vitamin E, amino acidand fatty acid composition of *Terminalia sericea. South African Journal of Botany* 88: 96-100

Choi SH and Ginsberg HN (2011). Increased very low density lipoprotein (VLDL) secretion, hepatic steatosis, and insulin resistance. *Trends in Endocrinology and Metabolism* 22: 353-363

Chung K, Wong TY, Wei C, Huang Y and Lin Y (1998). Tannins and human health: A Review. *Critical Reviews in Food Science and Nutrition* 38: 421-464

Cindoruk M, Kerem M, Karakan T, Salman B, Akin O, Alper M, Erdem O and Ünal S (2007). Peroxisome proliferators-activated alpha agonist treatment ameliorates hepatic damage in rats with obstructive jaundice: an experimental study. *Bio Med Central Gastroenterology* 44: 1-7

Coetzee PP, Coetzee LL, Puka R and Mubenga S (2003). Characterisation of selected South African clays for defluoridation of natural waters. *Water SA* 28: 331-338

Dachani SR, Ananth PH, Avanapu SR and Ibrahim M (2012). Preventive effect of *Anogeissus latifolia* in high fructose diet induced insulin resistance and metabolic dyslipidemia. *Journal of Natural Sciences Research* 2: 121-132

Davidson BC (1998). Seasonal changes in leaf lipid and fatty acid composition of nine plants consumed by two African herbivores. *Lipids* 33: 109-113

de Castro UGM, dos Santos RAS, Silva ME, de Lima WG, Campagnole-SantosMJ and Alzamora AC (2013). Age-dependent effect of high-fructose and high-fat diets on lipid metabolism and lipid accumulation in liver and kidney of rats. *Lipids in Health and Disease* 12: 136

de Moura RF, Ribeiro C, de Oliveira JA, Stevanato E and de Mello MAR (2009). Metabolic syndrome signs in Wistar rats submitted to different high-fructose ingestion protocols. *British Journal of Nutrition* 101: 1178-1184

de Paula FJA and Rosen CJ (2010). Back to the future: Revisiting parathyroid hormone and calcitonin control of bone remodeling. *Hormone and Metabolic Research* 42: 299-306

Dentin R, Liu Y, Koo SH, Hedrick S, Vargas T, Heredia J, Yates J and Montminy M (2007). Insulin modulates gluconeogenesis by inhibition of the coactivator TORC2. *Nature* 449: 366-370

Deutschländer MS, Lall N and van de Venter M (2009). Plant species used in the treatment of diabetes by South African traditional healers: An inventory. *Pharmaceutical Biology* 47: 348-365

Din N, Dibong SD, Mpondo E, Priso RJ, Kwin NF and Ngoye A (2011). Inventory and Identification of Plants Used in the Treatment of Diabetes in Douala Town (Cameroon). *European Journal of Medicinal Plants* 1: 60-73

Donaghue KC, Chiarelli F, Trotta D, Allgrove J and Dahl-Jorgensen K (2009). Microvascular and macrovascular complications associated with diabetes in children and adolescents. *Pediatric Diabetes* 10: 195-203

Doss A (2009). Preliminary phytochemical screening of some Indian medicinal plants. *Ancient Science of Life* 29: 12-16

Duhaney TS, Cui L, Rude MK, Lebrasseur NK, Ngoy S, De Silva DS, Siwik DA, Liao R and Sam F (2007). Peroxisome proliferator-activated receptor α–independent actions of fenofibrate exacerbates left ventricular dilation and fibrosis in chronic pressure overload. *Hypertension* 49: 1084-1094

Dwivedi S and Agarwal MP (1994). Antianginal and cardioprotective effects of *Terminalia arjuna*, an indigenous drug, in coronary artery disease. *The Journal of the Association of Physicians of India* 42: 287-289

Dwivedi S (2007). *Terminalia arjuna* Wight & Arn.-A useful drug for cardiovascular disorders. *Journal of Ethnopharmacology* 114: 114-129

Eckel RH, Grundy SM and Zimmet PZ (2005). The metabolic syndrome. *Lancet* 365: 1415-1428

Eldeen IMS, Elgorashi EE, Mulholland DA and van Staden J (2006). Anolignan B: A bioactive compound from the roots of *Terminalia sericea*. *Journal of Ethnopharmacology* 103: 135-138

Eldeen IMS, Elgorashi EE and van Staden J (2005). Antibacterial, anti-inflammatory, anti-cholinesterase and mutagenic effects of extracts obtained from some trees used in South Africantraditional medicine. *Journal of Ethnopharmacology* 102: 457-464

Eldeen IMS, Van Heerden FR and van Staden J (2008). Isolation and biological activities of termilignan b and arjunic acid from *Terminalia sericea* roots. *Planta Medica* 74: 411-413

Eloff JN, Katerere DR and McGaw LJ (2008). The biological activity and chemistry of the southern African Combretaceae. *Journal of Ethnopharmacology* 119: 686-699

Erasto P, Adebola PO, Grierson DS and Afolayan AJ (2005). An ethnobotanical study of plants used for the treatment of diabetes in the Eastern Cape Province. South Africa. *African Journal of Biotechnology* 4: 1458-1460

Ferreira AVM, Parreira GG, Porto LCJ, Mario EG, Delpuerto HL, Martins AS, Botion LM (2008). Fenofibrate prevents orotic acid-Induced hepatic steatosis in rats. *Life Sciences* 82: 876-883

Florez H, Temprosa MG, Orchard TJ, Mather KJ, Marcovina SM, Barrett-Connor E, Horton E, Saudek C, Pi-Sunyer XF Ratner RE and Goldberg RB (2013). Metabolic syndrome components and their response to lifestyle and metformin interventions are associated with differences in diabetes risk in persons with impaired glucose tolerance. *Diabetes, Obesity and Metabolism* DOI: 10.1111/dom.12220

Francis G, Kerem Z, Makkar HPS and Becker K (2002). The biological action of saponins in animal systems: A review. *British Journal of Nutrition* 88: 587-605

Friend A, Craig L and Turner S (2013). The prevalence of metabolic syndrome in children:A Systematic Review of the Literature. *Metabolic Syndrome and Related Disorders* 11: 71-80

Furuhashi M, Ura N, Murakami H, Hyakukoku M, Yamaguchi K, Higashiura K and **Shimamoto K (2002).** Fenofibrate improves insulin sensitivity in connection with intramuscular lipid content, muscle fatty acid-binding protein, and β-oxidation in skeletal muscle. *Journal of Endocrinology* 174: 321-329

Fyhrquist P, Mwasumbi L, Hæggstrom CA, Vuorela H, Hiltunen R and Vuorela P (2002). Ethnobotanical and antimicrobial investigation on some species of *Terminalia* and

Combretum (Combretaceae) growing in Tanzania. *Journal of Ethnopharmacology* 79: 169-177

Fyhrquist P, Mwasumbi L, Vuorela P, Vuorela H, Hiltunen R, Murphy C and Adlercreutz H (2006). Preliminary antiproliferative effects of some species of *Terminalia, Combretum* and Pteleopsis collected in Tanzania on some human cancer cell lines. *Fitoterapia* 77: 358-366

Gall WE, Beebe K, Lawton KA, Adam KP, Mitchell MW, Nakhle PJ, Ryals JA, Milburn MV, Nannipieri M, Camastra S, Natali A, Ferrannini E (2010). α-Hydroxybutyrate is an early biomarker of insulin resistance and glucose intolerance in a nondiabeticpopulation. *Plos One*5: e10883. doi:10.1371/journal.pone.0010883 10883

Gasa Z (2012). Effects of crude Aloe vera leaf extracts on diet-induced metabolic dysfunction in growing rats. MSc Dissertation, University of Witwatersrand

Gauthaman K, Banerjee SK, Dinda AK, Ghosh CC and Maulik SK (2005). *Terminalia arjuna* (Roxb.) protects rabbit heart against ischemic-reperfusion injury: role of antioxidant enzymes and heat shock protein. *Journal of Ethnopharmacology* 96: 403-409

Gauthaman K, Maulik M, Kumari R, Manchanda S.C, Dinda AK and Maulik SK (2001). Effect of chronic treatment with bark of *Terminalia arjuna*: a study on the isolated ischemic-reperfused rat heart. *Journal of Ethnopharmacology* 75: 197-201

Gersch MS, Mu W, Cirillo P, Reungjui S, Zhang L, Roncal C, Sautin YY, Johnson RJ, and Nakagawa T (2007). Fructose, but not dextrose, accelerates the progression of chronic kidney disease. *American Journal of Physiology - Renal Physiology* 293: F1256-F1261

Ghezzi AC, Cambri LT, Botezelli JD, Ribeiro C, Dalia RA and de Mello MAR (2012). Metabolic syndrome markers in wistar rats of different ages. *Diabetology and Metabolic Syndrome* 4: 1-16

González-Alvarado JM, Jime´nez-Moreno E, La´zaro R and Mateos GG (2007). Effect of type of cereal, heat processing of the cereal, and inclusion of fiber in the diet on productive performance and digestive traits of broilers. *Poultry Science* 86: 1705-1715

Govind P (2011). Medicinal plants against liver disease. *International Research Journal of Pharmacy* 2: 115-121

Gutman A and Shafrir E (1964). Metabolic influences on enzymes of glycogen synthesis and breakdown in adipose tissue. *American Journal of Physiology* 206: 1215-1220

Hajdu D, Aiglova K, Vinklerova I and Urbánek K (2009). Acute cholestatic hepatitis induced by fenofibrate. *Journal of Clinical Pharmacy and Therapeutics* 34: 599-602

Hagerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW and Riechel TL (1998). High molecular weight plant polyphenolics (tannins) as biological antioxidants. *Journal of Agricultural Food Chemistry* 46: 1887-1892

Han PW, Lin C, Chu K, Mu J and Liu A (1965). Hypothalamic obesity in weanling rats. *American Journal of Physiology* 209: 627-631

Harano Y, Yasui K, Toyama T, Nakajima T, Mitsuyoshi H, Mimani M, Hirasawa T, Itoh Y and Okanoue T (2006). Fenofibrate, a peroxisome proliferator-activated receptor α agonist, reduces hepatic steatosis and lipid peroxidation in fatty liver Shionogi mice with hereditary fatty liver. *Liver International* 26: 613-620

Hasenah A, Houghton PJ and Soumyanath A (2006). α-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to *Phyllanthus amarus. Journal of Ethnopharmacology* 107: 449-455

Herold KC, Hagopian W, Auger JA, Poumian-Ruiz E, Taylor L, Donaldson D, Gitelman SE, Harlan DM, Xu D, Zivin RA and Bluestone JA (2002). Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. *The New England Journal of Medicine*346: 1692-1698

Ho CY, Kuo TH, Chen TS, Tsay SH, Chang FY and Lee SD (2004). Fenofibrate-induced acute cholestatic hepatitis. *Journal of the Chinese Medical Association* 67: 245-247

Hong XZ, Li LD and Wu LM (2007). Effects of fenofibrate and xuezhikang on high-fat diet-induced non-alcoholic fatty liver disease. *Clinical and Experimental Pharmacology and Physiology* 34: 27-35

Hotamisligil GS, Murray DL, Choy LN and Spiegelman BM (1994). Tumor necrosis factor alpha inhibits signalling from the insulin receptor. *Proceedings of the National Academy of Sciences* 91: 4854-4858

Hsu C, Sun S, Lin S, Huang H and Wong K (2012). Moderate glucose control results in less negative nitrogen balances in medical intensive care unit patients: a randomized, controlled study. *Critical Care* doi:10.1186/cc11299

Hu W, Liu J, Wu Y, Guo Y and Ye J (2006*).* Effects of tea saponins on in vitro ruminal fermentation and growth performance in growing Boer goat. *Archives of Animal Nutrition* 60: 89-97

Huang B-W, Chiang M-T, Yao H-T and Chiang W (2004). The effect of high-fat and high-fructose diets on glucose tolerance and plasma lipid and leptin levels in rats. *Diabetes, Obesity and Metabolism* 6: 120-126

Hurst RT and Lee RW (2003). Increased incidence of coronary atherosclerosis in type 2 diabetes mellitus: mechanisms and management. *Annals of Internal Medicine* 139: 824-834

Inzucchi SE and Sherwin RS (2011). Goldman: Goldman's Cecil Medicine, 24th ed. Elsevier

Jellinger PS (2007). Metabolic consequences of hyperglycemia and insulin resistance. *Insulin* 8: S30–S42

Jeong S, Han M, Lee H, Kim M, Kim J, Nicol CJ, Kim BH, Choi JH, Nam K, Oh GT and Yoon M (2004)a. Effects of fenofibrate on high-fat diet–induced body weight gain and adiposity in female C57BL/6J mice. *Metabolism* 53: 1284-1289

Jeong S, Kim M, Han M, Lee H, Ahn J, Kim M, Song Y, Shin C, Nam K, Kim TW, Oh GT and Yoon M (2004)b. Fenofibrate prevents obesity and hypertriglyceridemia in low-density lipoprotein receptor-null mice. *Metabolism* 53: 607-613

Jialal I and Smith G (2012). Managing the dyslipidemia of metabolic syndrome:beyond statin therapy. *Metabolic Syndrome and Related Disorders* 10: 159-160

Joseph CC, Moshi MJ, Innocent E and Nkunya MHH (2007). Isolation of a stilbene glycoside and other constituents of *Terminalia sericeae. African Journal of Traditional, Complementary and Alternative Medicines* 4: 383-386

Kamerman PR, Modisa BME and Mphahlele NR (2004). Artovastatin, a potent HMG-CoA reductase inhibitor, is not antipyretic in rats. *Journal of Thermal Biology* 29: 431-435

Kane JA, Kane SP and Jain S (1995). Hepatitis induced by traditional Chinese herbs; possible toxic components. *Gut* 36: 146-147

Kanuri G, Spruss A, Wagnerberger S, Bischoff SC and Bergheim I (2011). Fructose-induced steatosis in mice: role of plasminogen activator inhibitor-1, microsomal triglyceride transfer protein and NKT cells. *Laboratory Investigation* 91: 885-895

Kelishadi R (2007). Childhood overweight, obesity, and the metabolic syndrome in developing countries. *Epidemiologic Reviews* 29: 62-76

Kirpichnikov D, McFarlane SI and Sowers JR (2002). Metformin: An update. *Annals of Internal Medicine* 137: 25-33

Kochhar A, Nagi M and Sachdeva R (2007). Effect of supplementation of traditional medicinal plants on serum lipid profile in non-insulin dependent diabetics. *Journal of Human Ecology* 22: 35-40

Kohen-Avramoglu R, Theriault A and Adeli K (2003). Emergence of the metabolic syndrome in childhood: an epidemiological overview and mechanistic link to dyslipidemia. *Clinical Biochemistry* 36: 413-420

Kraja AT, Province MA, Straka RJ, Ordovas JM, Borecki IB and Arnett DK (2010). Fenofibrate and metabolic syndrome. *Endocrine, Metabolic & Immune Disorders – Drug Targets* 10: 138-148

Kumar GPS, Arulselvan P, Kumar DS and Subramanian SP (2006). Anti-diabetic activity of fruits *of Terminalia chebula* on streptozotocin induced diabetic rats. *Journal of Health Science* 52: 283-291

LaRosa JC, Pedersen TR, Somaratne R and Wasserman SM (2013). Safety and effect of very low levels of low-density lipoprotein cholesterol on cardiovascular events. *American Journal of Cardiology* 111: 1221-1229

Latha RCR and Daisy P (2010). Influence of *Terminalia bellerica* Roxb. Fruit Extracts on Biochemical Parameters in Streptozotocin Diabetic Rats. *International Journal of Pharmacology* 6: 89-96

Le K, Faeh D, Stettler R, Ith M, Kreis R, Vermathen P, Boesch C, Ravussin E and Tappy L (2006). A 4-wk high-fructose diet alters lipid metabolism without affecting insulin sensitivity or ectopic lipids in healthy humans. *American Journal of Clinical Nutrition* 84: 1374 -1379

Le K, Ith M, Kreis R, Faeh D, Bortolotti M, Tran C, Boesch C and Tappy L (2009). Fructose overconsumption causes dyslipidemia and ectopic lipid deposition in healthy subjects with and without a family history of type 2 diabetes. *American Journal of Clinical Nutrition* 89: 1760-1765

Lehnen AM, Rodrigues B, Irigoyen MC, De Angelis K and Schaan BD (2013). Cardiovascular changes in animal models of metabolic syndrome. *Journal of Diabetes Research* http://dx.doi.org/10.1155/2013/761314

Legendre C, Causse E, Chaput E, Salvayre R, Pineau T and Edgar AD (2002). Fenofibrate induces a selective increase of protein-bound homocysteine in rodents: a PPARα-mediated effect. *Biochemical and Biophysical Research Communications* 296: 1052-1056

Leng SH, Lu FE and Xu LJ (2004). Therapeutic effects of berberine in impaired glucose tolerance rats and its influence on insulin secretion. *Acta Pharmacologia Sinica* 25: 496-502

Lenzen S (2008). The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia* 51: 216-226

Levin A, Bakris GL, Molitch M, Smulders M, Tian J, Williams LA and Andress DL (2007). Prevalence of abnormal serum vitamin D, PTH, calcium, and phosphorus in patients with chronic kidney disease: Results of the study to evaluate early kidney disease. *Kidney International* 71: 31-38

Li C, Ford ES, McBride PE, Kwiterovich PO, McCrindle BW and Gidding SS (2011). Non–high-density lipoprotein cholesterol concentration is associated with the metabolic syndrome among us youth aged 12-19 years. *Journal of Pediatrics* 158: 201-207

Ling H, Luomab JT and Hilleman D (2013). A review of currently available fenofibrate and fenofibric acid formulations.*Cardiology Research* 4: 47-55

Liu S, Liu Q, Li L, Huan Y, Sun S and Shen Z (2011). Long-term fenofibrate treatment impaired glucose-stimulated insulin secretion and upregulated pancreatic NF-kappa B and iNOS expression in monosodium glutamate-induced obese rats: Is that a latent disadvantage? *Journal of Translational Medicine* 9: 2-11

London E and Castonguay TW (2011). High fructose diets increase 11β-hydroxysteroid dehydrogenase type 1 in liver and visceral adipose in rats within 24-h exposure. *Obesity* 19: 925-932

Loxham SJG, Teague J, Poucher SM, De Schoolmeester J, Turnbull AV and Carey F (2007). Glucagon challenge in the rat: a robust method for the vivo assessment of glycogen phosphorlyase inhibitor efficacy. *Journal of Pharmacology Toxicology Methods* 55: 71-77

Mahato SB, Sarkar SK and Poddar G (1988). Triterpenoid saponins. *Phytochemistry.* 27: 3037-3067

Manna P, Sinha M and Sil PC (2006). Aqueous extract of *Terminalia arjuna* prevents carbon tetrachloride induced hepatic and renal disorders. *BMC Complementary and Alternative Medicine* 6: 1-10

Masoko P, Picard J and Eloff JN (2005). Antifungal activities of six South African *Terminalia* species (Combretaceae)**.** *Journal of Ethnopharmacology* 99: 301-308

Masoko P and Eloff JN (2007). Screening of twenty-four South African *Combretum* and six *Terminalia* species (Combretaceae) for antioxidant activities. *African Journal of Traditional Complementary and Alternative Medicine* 4: 231-239

Mather KJ, Steinberg HO, Baron AD (2013). Insulin resistance in the vasculature. *The Journal of Clinical Investigation* 123: 1003-1004

Matsudaa M and Shimomurab I (2013). Increased oxidative stress in obesity: Implications for metabolic syndrome, diabetes, hypertension, dyslipidemia, atherosclerosis, and cancer. *Obesity Research & Clinical Practice* 7: e330-e341

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF and Turner RC (1985). Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentration in man. *Diabetologia* 28: 412-419

Mbanya JCN, Motala AA, Sobngwi E, Assah FK and Enoru ST (2010). Diabetes in sub-Saharan Africa. *Lancet* 375: 2254-2266

Metges CC (2011). Classical and post-genomic methods to study GIT function with emphasison the pig. *Livestock Science* 133: 10-19

Miller A and Adeli K (2008). Dietary fructose and the metabolic syndrome. *Current Opinion in Gastroenterology* 24: 204-209

Misra A and Khurana L (2008). Obesity and the metabolic syndrome in developing countries. *Journal of Clinical Endocrinology Metabolism* 93: S9-S30

M'marete CK (2003). Climate and water resources in the Limpopo Province.In: Nesamvuni AE, Oni SA, Odhiambo JJOand Nthakheni ND (eds.) Agriculture as the cornerstone of the economy in the Limpopo Province. A study commissioned by the economic cluster of the Limpopo provincial government under the leadership of the department of agriculture. 1-49 http://www.lda.gov.za (Accessed: 08.09.2013)

Mochizuki M and Hasegawa N (2006). Acceleration of lipid degradation by sericoside of *Terminalia sericea* roots in fully differentiated 3T3-L1 cells. *Phytotherapy Research* 20: 1020-1021

Mochizuki M and Hasegawa N (2007). Anti-inflammatory effect of extract of *Terminalia sericea* roots in an experimental model of colitis. *Journal of Health Science* 53: 329-331

Mollentze WF (2006). Obesity in South Africa: A call for action. *Journal of Endocrinology, Metabolism and Diabetes of South Africa* 9: 44-45

Morton NM, Densmore V, Wamil M, Ramage L, Nichol K, Bunger L, Seckl JR and Kenyon CJ (2005).A polygenic model of the metabolic syndrome withreduced circulating and intra-adipose glucocorticoidaction. *Diabete*s 54: 3371-3378

Moshi MJ and Mbwambo ZH (2005). Some pharmacological properties of extracts of *Terminalia sericea* roots. *Journal of Ethnopharmacology* 97: 43-47

Muhammad NO and Oloyede OB (2004). Assessment of biological value of *Terminalia catappa* seed meal-based diet in rats. *Biokemistri* 16:49-55

Muhammad S, Khan BA, Akhtar N, Mahmood T, Rasul A, Hussain I, Hand K and Badshah A (2012). The morphology, extractions, chemical constituents and uses of *Terminalia chebula:* A review. *Journal of Medicinal Plants Research* 6: 4772-4775

Murali YK, Chandra R and Murthy PS (2004). Antihyperglycemic effect of water extract of dry fruits of *Terminalia chebula* in experimental diabetes mellitus. *Indian Journal of Clinical Biochemistry* 19: 202-204

Nagappa AN, Thakurdesai PA, Rao NV and Singh J (2003). Antidiabetic activity of *Terminalia catappa Linn* fruits. *Journal of Ethnopharmacology* 88: 45-50

Neergheen-Bhujun VS, Rosun H, Joonas N & Bahorun T (2013). Antioxidant status of Mauritian subjects with type II diabetes mellitus. *International Journal of Diabetes in Developing Countries* DOI 10.1007/s13410-013-0129-z

Neuschwander-Tetri BA and Wang DQ-H (2013). Excess cholesterol and fat in the diet: a dangerous liaison for energy expenditure and the liver. *Hepatology* 57: 7-9

Njomen GBSN, Kamgang R, Soua PRN, Oyono JLE and Njikam N (2009). Protective effect of methanol-methylene chloride extract of *Terminalia glaucescens* leaves on

streptozotocin-induced diabetes in mice. *Tropical Journal of Pharmaceutical Research* 8: 19-26

Nkobole N, Houghton PJ, Hussein A and Lall N (2011). Antidiabetic activity of *Terminalia sericea* constituents. *Natural Product Communications* 6: 1-4

Oettl K, Birner-Gruenberger R, Spindelboeck W, Stueger HP, Dorn L, Stadlbauer V, Putz-Bankuti C, Krisper P, Graziadei I, Vogel W, Lackner C and Stauber RE (2013). Oxidative albumin damage in chronic liver failure: Relation to albumin binding capacity, liver dysfunction and survival. *Journal of Hepatology* 59: 978-983

Oron-Herman M, Kamari Y, Grossman E, Yeger G, Peleg E, Shabtay Z, Shamiss A and Sharabi Y (2008). Metabolic syndrome: comparison of the two commonly used animal models. *American Journal of Hypertension* 21: 1018-1022

Owens FN, Gill DR, Secrist DS and Coleman SW (1995). Review of some aspects of growth and development of feedlot cattle. *Journal of AnimalScience* 73: 3152-3172

Panchal SK and Brown L (2011). Rodent models for metabolic syndrome research. *Journal of Biomedicine and Biotechnology* doi:10.1155/2011/351982

Park CW, Kim HW, Ko SH, Chung HW, Lim SW, Yang CW, Chang YS, Sugawara A, Guan Y and Breyer MD (2006). Accelerated diabetic nephropathy in mice lacking the peroxisome proliferator-activated receptor α. *Diabetes* 55: 885-893

Passoneau JV and Lauderdale VR (1974). A comparison of three methods of glycogen measurement in tissues. *Analytical Biochemistry* 60: 405-12

Pérez YY, Jiménez-Ferrer E, Zamilpa A, Hernández-Valencia M, Alarcón-Aguilar FJ, Tortoriello J and Román-Ramos R (2007). Effect of a polyphenol-rich extract from *Aloe vera* gel on experimentally induced insulin resistance in mice. *The American Journal of Chinese Medicine* 35: 1037-1046

Pettitt DJ, Talton J, Dabelea D, Divers J, Imperatore G, Lawrence JM, Liese AD, Linder B, Mayer-Davis EJ, Pihoker C, Saydah SH, Standiford DA, Hamman RF (2013). Prevalence of diabetes mellitus in U.S. youth in 2009: The SEARCH for diabetes in youth study. *Diabetes Care* doi: 10.2337/dc13-1838

Pieper-Bigelow C, Strocchi A and Levitt MD (1990). Where does serum amylase come from and where does it go? *Gastroenterology Clinics of North America* 19: 793-810

Pratt DS and Kaplan MM (2000). Evaluation of abnormal liver-enzyme results in a symptomatic patients. *The New England Journal of Medicine* 342: 1266-71

Price SC, Hinton RH, Mitchell FE, Hall DE, Grasso P, Blane GF and Bridges JW (1986). Time and dose study on the response of rats to the hypolipidaemic drug fenofibrate. *Toxicology* 41: 169-191

Qu F, Chen Z, Wang X, Meng L, Wu Z and Qu A (2011). Observing GLUT4 translocation in live L6 cells using quantum dots. *Sensors* 11: 2077-2089

Racine NM, Watras AC, Carrel AL, Allen DB, McVean JJ, Clark RR, O'Brien AR, O'Shea M, Scott CE and Schoeller DA (2010). Effect of conjugated linoleic acid on body fat accretion in overweight obese children. *American Journal of Clinical Nutrition* 91: 1157-1164

Raghavan B and Kumari SK (2008). Effect of *Terminalia arjuna* stem bark on antioxidant status in liver and kidney of alloxan diabetic rats. *Indian Journal of Physiology and Pharmacology* 50: 133-142

Ram A, Lauria P, Gupta R, Kumar P and Sharma VN (1997). Hypocholesterolaemic effects of *Terminalia arjuna* tree bark. *Journal of Ethnopharmacology* 55: 165-169

Rao NK and Nammi S (2006). Antidiabetic and renoprotective effects of the chloroform extract of *Terminalia chebula* Retz. seeds in streptozotocin-induced diabetic rats. *BMC Complementary and Alternative Medicine* 6: 17

Reichling JJ and Kaplan MM (1988). Clinical use of serum enzymes in liver disease. *Digestive Diseases and Sciences* 33: 1601-1614

Reyes-Gordillo K, Segovia J, Shibayama M, Vergara P, Moreno MG and Muriel P (2007). Curcumin protects against acute liver damage in the rat by inhibiting NF-κB, proinflammatory cytokines production and oxidative stress. *Biochimicaet Biophysica Acta* 1770: 989-996

Rode T, Frauen M, Muller BW, Dusingc HJ, Schonrock U, Mundt C and Wenck H (2003). Complex formation of sericoside with hydrophilic cyclodextrins: improvement of solubility and skin penetration in topical emulsion based formulations. *European Journal of Pharmaceutics and Biopharmaceutics* 55: 191-198

Rol De Lama MA, Pe´rez-Romero A, Tresguerres JAF, Hermanussen M and Ariznavarreta C (2000). Recombinant human growth hormone enhances tibial growth in peripubertal female rats but not in males. *European Journal of Endocrinology* 142: 517-523

Rotimi C, Daniel H, Zhou J, Obisesan A, Chen G, Chen Y, Amoah A, Opoku V, Acheampong J, Agyenim-Boateng K, EghanJr BA, Oli J, Okafor G, Ofoegbu E, Osotimehin B Abbiyesuku F, Johnson T, Fasanmade O, Doumatey A, Aje T, Collins F and Dunston G (2003). Prevalence and determinants of diabetic retinopathy and cataracts in West African type 2 diabetes patients. *Ethnicity and Disease* 13: 110-117

Rubino F, **R'bibo SL**, **del Genio F**, **Mazumdar M and McGraw TE (2010).** Metabolic surgery: the role of the gastrointestinal tract in diabetes mellitus. *Natural Review: Endocrinology* 6: 102-109

Russell JC and Proctor SD (2006). Small animal models of cardiovascular disease: tools for the study of the roles of metabolic syndrome, dyslipidemia, and atherosclerosis. *Cardiovascular Pathology* 15: 318-330

Rutledge AC and Adeli K (2007). Fructose and the metabolic syndrome: pathophysiology and molecular mechanisms. *Nutrition Reviews* 65: 13-23

Sallie R, Tredger JM and Williams R (1991). Review article drugs and the liver Part 1: testing liver function. *Biopharmaceutics and Drug Disposition* 12: 251-259

Sanchez CP and He Y (2009). Bone growth during rapamycin therapy in young rats. *BMC Pediatrics* 9: 3

Sánchez-Lozada LG, Tapia E, Jime´nez A, Bautista P, Cristobal M, Nepomuceno T, Soto V, Avila-Casado C, Nakagawa T, Johnson RJ, Herrera-Acosta J and Franco M (2007). Fructose-induced metabolic syndrome is associated with glomerular hypertension and renal microvascular damage in rats. *American Journal of Physiology Renal Physiology* 292: F423-F429

Sandhar HK, Bimlesh K, Prasher S, Tiwari P, Salhan M and Sharma P (2011). A review of phytochemistry and pharmacology of flavonoids. *Internationale Pharmaceutica Sciencia* 1: 25-41

Scalbert A (1991). Antimicrobial properties of tannins. *Phytochemisrry* 30: 3875-3883,

Scheepers A, Joost HG and Schurmann A (2004). The glucose transporter families SGLT and GLUT: molecular basis of normal and aberrant function. *Journal of Parenteral and Enteral Nutrition* 28: 364-371

Shackleton CM (2001). Managing regrowth of an indigenous savanna tree species (*Terminalia sericea*) for fuelwood: the influence of stump dimensions and post-harvest coppice pruning. *Biomass and Bioenergy* 20: 261-270

Singh G, Singh AT, Abraham A, Bhat B, Mukherjee A, Verma R, Agarwal SK, Jha S, Mukherjee R and Burman AC (2008). Protective effects of *Terminalia arjuna* against Doxorubicin-induced cardiotoxicity. *Journal of Ethnopharmacology* 117: 123-129

Singh GK (2006). Metabolic syndrome in children and adolescents. *Current treatment options in cardiovascular medicine*. 8: 403-413.

Sinha M, Manna P and Sil PC (2008). *Terminalia arjuna* protects mouse hearts against sodium Fluoride-Induced Oxidative Stress. *Journal of Medicinal Food* 11: 733-740

Song M, Schuschke DA, Zhou Z, Chen T, Shi X, Zhang J, Zhang X, Pierce JrWM, Johnson WT, Vos MB and McClain CJ (2013). Modest Fructose beverage intake causes liver injury and fat accumulation in marginal copper deficient rats. *Obesity* 21: 1669-1675

Southerland JH, Moss K, Taylor GW, Beck JD, Pankow J, Gangula PR and Offenbacher S (2012). Periodontitis and diabetes associations with measures of atherosclerosis and CHD. *Atherosclerosis* 222: 196-201

Subramaniam S, Subramaniam R, Rajapandian S, Uthrapathi S, Gnanamanickam RV and Dubey GP (2011). Anti-atherogenic activity of ethanolic fraction of *Terminalia arjuna* bark on hypercholesterolemic rabbits. *Evidence-Based Complementary and Alternative Medicine* doi:10.1093/ecam/neq003

Suchalatha S and Shyamala DCS (2004). Protective effect of *Terminalia chebula* against experimental myocardial injury induced by isoproterenol. *Indian Journal of Experimental Biology* 42: 174-178

Staels B, Dallongeville J, Auwerx J, Schoonjans K, Leitersdorf E and Fruchart J. (1998). Mechanism of action of fibrates on lipid and lipoprotein metabolism. *Circulation* 98: 2088-2093

SteenkampV, Mathivha E, Gouws MC and van Rensburg CEJ (2004). Studies on antibacterial, antioxidant and fibroblast growth stimulation of wound healing remedies from South Africa. *Journal of Ethnopharmacology* 95: 353-357

Steinmetz J, Morin C, Panek E, Siest G and Drouin P (1981). Biological variations in hyperlipidemic children and adolescents treated with fenofibrate. *Clinica Chimica Acta.* 112: 43-53

Steyn NP and Temple NJ (2012). Evidence to support a food-based dietary guideline on sugar consumption in South Africa. *BMC Public Health* 12: 502-510

Syversen U, Stunes AK, Gustafsson BI, Obrant KJ, Nordsletten L, Berge R, Thommesen L and Reseland JE (2009). Different skeletal effects of the peroxisome proliferator activated receptor (PPAR)α agonist fenofibrate and the PPARγ agonist pioglitazone. *BMC Endocrine Disorders* 9: 1-13

Szkudelski T (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiological Research* 50: 536-546

Tanaka Y, Kume S, Araki S, Isshiki K, Chin-Kanasaki M, Sakaguchi M, Sugimoto T, Koya D, Haneda M, Kashiwagi A, Maegawa H and Uzu T (2011). Fenofibrate, a PPARa agonist, has renoprotective effects in mice by enhancing renal lipolysis. *Kidney International* 79: 871-882

Tapas AR, Sakarkar DM and KakdeRB (2008). Flavonoids as nutraceuticals: A review. *Tropical Journal of Pharmaceutical Research* 7: 1089-1099

Tasduq SA, Singh K, Satti NK, Gupta DK, Suri KA and Johri RK (2006). *Terminalia chebula* (fruit) prevents liver toxicity caused by sub-chronic administration of rifampicin, isoniazid and pyrazinamide in combination. *Human Experimental Toxicology* 25: 111-118

Taylor EN and Curhan GC (2008). Fructose consumption and the risk of kidney stones. *Kidney International* 73: 207-212

Thirunavukkarasu V, Nandhini ATA and Anuradha CV (2004). Effect of α-lipoic acid on lipid profile in rats fed a high-fructose diet. *Experimental Diabetes Research* 5: 195-200

Thulin P, Rafter I, Stockling K, Tomkiewicz C, Norjavaara E, Aggerbeck M, Hellmold H, Ehrenborg E, Andersson U, Cotgreave I and Glinghammar B (2008). PPARα regulates the hepatotoxic biomarker alanine aminotransferase (ALT1) gene expression in human hepatocytes. *Toxicology and Applied Pharmacology* 231: 1-9

Tiwari AK, Gode JD and Dubey GP (1990). Effect of *Terminalia Arjuna* on lipid profiles of rabbits fed hypercholesterolemic diet. *Pharmaceutical Biology* 28: 43-47

Tolman KG (2000). Defining patient risks from expanded preventive therapies. *The American Journal of Cardiology* 85:15E-19E

Toriola AL, Moselakgomo VK, Shaw BS and Goon DT (2012). Overweight, obesity and underweight in rural black South African children. *South African Journal of Clinical Nutrition* 25: 57-61

Tormo MA, Gil-Exojo I, de Tejada AR and Campillo JE (2004). Hypoglycaemic and an orexigenic activities of an α-amylase inhibitor from white kidney beans (*Phaseolus vulgaris*) in Wistar rats. *British Journal of Nutrition* 92: 785-790

Tshikalange TE, Meyer JJM, Lall N, Munoz E, Sancho R, Van de Venter M and Oosthuizen V (2008). In vitro anti-HIV-1 properties of ethnobotanically selected South African plants used in the treatment of sexually transmitted diseases. *Journal of Ethnopharmacology* 119: 478-481

Tsimihodimos V, Miltiadous G, Daskalopoulou SS, Mikhailidis DP and Elisaf MS (2005). Fenofibrate: Metabolic and pleiotropic effects. *Current Vascular Pharmacology* 3: 87-98

Uchida A, Slipchenko MN, Cheng J and Buhman KK (2011). Fenofibrate, a peroxisome proliferator-activated receptor α agonist, alters triglyceride metabolism in enterocytes of mice. *Biochimica et Biophysica Acta* 1811: 170-176

Valasek MA, Clarke SL and Repa JJ (2007). Fenofibrate reduces intestinal cholesterol absorption via PPARα-dependent modulation of NPC1L1 expression in mouse. *Journal of Lipid Research* 48: 2725-2735

Wajchenberg BL, Nery M, Cunha MR and da Silva MER (2009). Adipose tissue at the crossroads in the development of the metabolic syndrome, inflammation and atherosclerosis. *Arquivos Brasileiros de Endocrinologia and Metabologia* 53: 145-150

Wall ME,Wani MC, Brown DM, Fullas F, Olwald JB, Josephson FF, Thornton NM, Pezzuto JM, Beecher CWW, Farnsworth NR, Cordell GA andKinghorn AD (1996). Effect of tannins on screening of plant extracts for enzyme inhibitory activity and techniques for their removal. *Phytomedicine* 3: 281-285

Weiss R, Dziura J, Burgert TS, Tamborlane WV, Taksali SE, Yeckel CW, Allen K, Lopes M, Savoye M, Morrison J, Sherwin RS and Caprio S (2004). Obesity and themetabolic syndrome in children and adolescents. *The New England Journal of Medicine* 350: 2362-74

Wina E, Muetzel S and Becker K (2005). The impact of saponins or saponin-containing plant materials on ruminant productions: A Review. *Journal of Agricultural and Food Chemistry* 53: 8093-8106

Wilson RD and Islam S (2012). Fructose-fed streptozotocin-injected rat:an alternative model for type 2 diabetes. *Pharmacological Reports* 64: 129-139

Wolf PL (1999). Biochemical diagnosis of liver disease. *Indian Journal of Clinical Biochemistry.* 14: 59-90

Woo HM, Noack S, Seibold GM, Willbold S, Eikmanns BJ and Bott M (2010). Link between phosphate starvation and glycogen metabolism in *Corynebacterium glutamicum*, revealed by metabolomics. *Applied Environmental Microbiology* 76: 6910-6919

Wu G, Collins JK, Perkins-Veazie P, Siddiq M, Dolan KD, Kelly KA, Heaps CL and Meininger CJ (2007). Dietary supplementation with watermelon pomace juice enhances arginine availability and ameliorates the metabolic syndrome in zucker diabetic fatty rats. *The Journal of Nutrition* 137: 2680-2685

Yadav H, Jain S and Sinha PR (2007). Antidiabetic effect of probiotic dahi containing *Lactobacillus acidophilus* and *Lactobacillus casei* in high fructose fed rats. *Nutrition* 23: 62-68

Younes H, Coudray C, Bellanger J, Demigne C, Rayssiguier Y and Remesy C (2001). Effects of two fermentable carbohydrates (inulin and resistant starch) and their combination on calcium and magnesium balance in rats. *British Journal of Nutrition* 86: 479-485

Zabielski R, Godlewski MM and Guilloteau P (2008). Control of development of gastrointestinal system in neonates. *Journal of Physiology and Pharmacology* 59: 35-35

Zhang HJ, Guo YM, Tian YD and Yuan JM (2008)a. Dietary conjugated linoleic acid improves antioxidant capacityin broiler chicks. *British Poultry Science* 49: 213-221

Zhang M, Lv X-Y, Li J, Xu Z-G, and Chen L (2008)b. The characterization of high-fat diet and multiple low-dose streptozotocin induced type 2 diabetes rat model. *Experimental Diabetes Research* doi:10.1155/2008/704045 1-9

<http://www.betacell.org/content/articleview/article_id/1/> Beta Cell Biology Consortium, 2004. (Accssed 19.11.1013)

APPENDICES

Appendix 1

AESC3

STRICTLY CONFIDENTIAL

ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2012/29/05

APPLICANT: **Mr B W Lembede**

DEPARTMENT: **Physiology**

PROJECT TITLE: **Effect of dietary *Terminalia sericea* leaf aqueous extracts on the development of high-fructose diet-induced metabolic dysfunction in growing Wistar rats.**

Number and Species

40 Wistar Rats

Approval was given for the use of animals for the project described above at an AESC meeting held on **26 June 2012**. This approval remains valid until **30 June 2014**.

The use of these animals is subject to AESC guidelines for the use and care of animals, is limited to the procedures described in the application form and to the following additional conditions:

Conditions:

- A score sheet to be used to log the health of the rats.

Signed: _____ (Chairperson, AESC) Date: 31/7/12.

I am satisfied that the persons listed in this application are competent to perform the procedures therein, in terms of Section 23 (1) (c) of the Veterinary and Para-Veterinary Professions Act (19 of 1982)

Signed: _____ (Registered Veterinarian) Date: 31/7/12

cc: Supervisor:
Director: CAS

6 REAGENTS 1 X 96 KIT

Each High Range Rat Insulin ELISA kit contains reagents for 96 wells, sufficient for 42 samples and one standard curve in duplicate. For larger series of assays, use pooled reagents from packages bearing identical lot numbers.

The expiry date for the complete kit is stated on the outer label.

The recommended storage temperature is 2 °C - 8 °C.

Coated Plate	1 plate	96 wells	Ready for use
(mouse monoclonal anti-insulin)		8-well strips	

For unused microplate strips, reseal the bag using adhesive tape, store at 2–8°C and use within 8 weeks.

Calibrators 1, 2, 3, 4, 5	5 vials	1000 µL	Ready for use

Rat Insulin

Color coded yellow

Concentration stated on vial label.

Calibrator 0	1 vial	5 mL	Ready for use

Color coded yellow

Enzyme Conjugate 11X	1 vial	600 µL	Preparation, see below

Peroxidase conjugated mouse monoclonal anti-insulin

Enzyme Conjugate Buffer	1 vial	6 mL	Ready for use

Color coded blue

Wash Buffer 21X	1 bottle	50 mL	Dilute with 1000 mL redistilled water to make wash buffer 1x solution

Storage after dilution: 2 °C - 8 °C for 8 weeks

Substrate TMB	1 vial	22 mL	Ready for use

Colorless solution *Note! Light sensitive!*

Stop Solution	1 vial	7 mL	Ready for use

0.5 M H_2SO_4

6.1 Preparation of enzyme conjugate 1X solution

Prepare the needed volume of enzyme conjugate 1X solution by dilution of Enzyme Conjugate 11X (1+10) in Enzyme Conjugate Buffer according to the table below.

When preparing enzyme conjugate 1X solution for the whole plate, pour all of the Enzyme Conjugate Buffer into the Enzyme Conjugate 11x vial. Mix gently.

Number of strips	Enzyme Conjugate 11X	Enzyme Conjugate buffer
12 strips (1 plate)	1 vial	6 mL
6 strips	300 µL	3 mL
4 strips	200 µL	2 mL

Storage after dilution: 2 °C - 8 °C for 8 weeks.

7 SPECIMEN COLLECTION AND HANDLING

Serum

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation.

Samples can be stored at 2 °C - 8 °C up to 24 hours.

For longer periods store samples at -20 °C. Avoid repeated freezing and thawing.

Plasma

Collect blood by venipuncture into tubes containing heparin or EDTA as anticoagulant, and separate the plasma fraction.

Samples can be stored at 2 °C - 8 °C up to 24 hours.

For longer periods store samples at -20 °C. Avoid repeated freezing and thawing.

7.1 Preparation of samples

No dilution is normally required, however, samples containing >150 µg/L should be diluted 1/10 v/v with Calibrator 0.

Note! Buffer containing sodium azid (NaN_3) can not de used for sample dilution.

8 TEST PROCEDURE

Prepare a calibrator curve for each assay run. All reagents and samples must be brought to room temperature before use.

1. Prepare enzyme conjugate 1X solution (according to the tables on previous pages), wash buffer 1X solution and samples.
2. Prepare sufficient microplate wells to accommodate Calibrators and samples in duplicate.
3. Pipette 10 µL each of Calibrators and samples into appropriate wells.
4. Add 50 µL of enzyme conjugate 1X solution into each well.
5. Incubate on a plate shaker (700-900 rpm) for 2 hours at room temperature (18 °C – 25 °C).
6. Wash 6 times with 700 µL wash buffer 1X solution per well using an automatic plate washer with overflow-wash function. Do not include soak step in washing procedure.
   Or manually,
   Discard the reaction volume by inverting the microplate over a sink. Add 350µL wash buffer 1X solution to each well. Discard the wash solution, tap firmly several times against absorbent paper to remove excess liquid. Repeat 5 times. Avoid prolonged soaking during washing procedure.
7. Add 200 µL Substrate TMB into each well.
8. Incubate 15 minutes at room temperature (18 °C – 25 °C).
9. Add 50 µL Stop Solution to each well.
   Place the plate on the shaker for approximately 5 seconds to ensure mixing.
10. Read optical density at 450 nm and calculate results.
    Read within 30 minutes.

Note! To prevent contamination between the conjugate and substrate, separate pipettes are recommended.

9 INTERNAL QUALITY CONTROL

Commercial controls and/or internal serum pools with low, intermediate and high insulin concentrations should routinely be assayed as unknowns, and results charted from day to day. It is good laboratory practice to record the following data for each assay: kit lot number; dilution and/or reconstitution dates of kit components, OD values for the blank, Calibrators and controls..