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THE EFFECTS OF CRUDE *FICUS THONNINGII* STEM-BARK EXTRACT ON HIGH-FRUCTOSE DIET FED GROWING SPRAGUE DAWLEY RATS

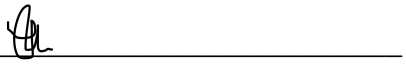
Yvonne Mhosva

A dissertation submitted to the Faculty of Health Sciences, University of Witwatersrand, School of Physiology in fulfilment of the requirements for the degree of Master of Science in Medicine.

Johannesburg, 2019

DECLARATION

I, **Yvonne Mhosva**, declare that this dissertation is my own unaided work. Where work from other sources has been used; it has been appropriately acknowledged. It is being submitted for the degree of Master of Science in Medicine at the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any degree or examination at any other University. I confirm that all the experimental procedures used in this dissertation were approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (AESC number: 2016/03/13/B).



Yvonne Mhosva

Signed on the 6th day of April 2020

DEDICATION

To my loving and supportive siblings,

To my supervisors, colleagues, and friends for the motivation and tireless support.

PRESENTATIONS ARISING FROM THIS STUDY

Yvonne Mhosva, Trevor Nyakudya and Eliton Chivandi (2018). Effect of *Ficus thonningii* stem-bark extract on liver mass, hepatic lipid content and plasma triglyceride levels in fructose-fed Sprague Dawley rats. The First Conference of Biomedical and Natural Sciences and Therapeutics (CoBNeST), Spier Conference Centre, Stellenbosch, South Africa, 7-12th October 2018.

ABSTRACT

The consumption of fructose-rich diets is one of the causes of the global increase in the prevalence of obesity and metabolic derangements (MD) in children. Pharmacological agents used to manage MD are expensive, inaccessible and elicit side effects. Communities, therefore, depend on plant-derived ethnomedicines for primary healthcare. *Ficus thonningii* extracts contain phytochemicals with hypoglycaemic, hypolipidaemic and anti-oxidant effects. I investigated the prophylactic potential of methanolic *F. thonningii* stem-bark extracts (MEFT) to protect against high-fructose diet (HFD) induced MD in growing Sprague Dawley (SD) rats mimicking children fed obesogenic diets.

Eighty 21-day old SD rat pups (40 males; 40 females) were randomly allocated and administered the following treatment regimens: group 1 – standard rat chow (SRC) + water (W); group 2 - SRC + 20% (w/v) fructose solution (FS); group 3: SRC + FS + fenofibrate at 100 mg/kg bwt/day (FEN); group 4 – SRC + FS + low dose MEFT (LDMEFT) at 50 mg/kg bwt/day and group 5 – SRC + FS + high dose MEFT (HDMEFT) at 500 mg/kg bwt/day for 8 weeks. Body mass was measured twice weekly. At the end of the 8-week experimental period, the rats were subjected to an oral glucose tolerance test. Forty-eight hours later, the rats were then fasted overnight and fasting blood glucose and triglyceride concentration and haematocrit were determined. Thereafter the rats were euthanised. Plasma insulin concentration and surrogate markers of health were determined. HOMA-IR was computed. Viscera macro- and micro-morphometry and hepatic lipid content were also determined.

Consumption of the HFD did not affect ($P>0.05$) body mass, tolerance to an oral glucose load, HOMA-IR, haematocrit, viscera macro-morphometry, plasma insulin concentration, ALT and ALP activities as well as plasma BUN, cholesterol and creatinine concentrations of the rats. The HFD increased ($P<0.05$) plasma triglyceride concentration ($P<0.05$) in the rats but decreased ($P<0.05$) the liver lipid content of female rats compared to counterparts administered the control treatment regimen. The LDMEFT increased ($P<0.05$) liver lipid content of female rats. The HFD caused micro-steatosis and hepatic inflammation ($P<0.05$) in male and female rats but caused macro-steatosis ($P<0.05$) in females only. FEN, LDMEFT and HDMEFT protected against steatosis and inflammation in female rats ($P<0.05$). In males, both the LDMEFT and HDMEFT protected against both steatosis and inflammation but FEN protected only against micro-steatosis. In male rats, the HFD decreased ($P<0.05$) long bone density. FEN, LDMEFT and HDMEFT prevented the HFD-induced increased plasma

triglyceride concentration. The HFD had no effect ($P>0.05$) on tibiae and femora indices of rats. FEN decreased ($P<0.05$) femora mass and density in males and the low dose MEFT decreased ($P<0.05$) femora density in females. The HDMEFT decreased ($P<0.05$) tibiae length of female rats. FEN increased ($P<0.05$) the liver mass in both rat sexes. The LDMEFT increased ($P<0.05$) liver lipid in female rats. FEN, LDMEFT and HDMEFT attenuated HFD diet-induced high plasma triglyceride concentration. The HFD elicited elements of MD in a sexually dimorphic manner. The crude MEFT stem bark extracts potentially could be used to prevent HDF diet-induced hypertriglyceridemia, hepatic steatosis and inflammation. It should be used with caution since it caused hepatic lipid accretion and compromised bone length and density in females.

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LIST OF ABBREVIATIONS

α:	Alpha
ALP:	Alkaline phosphatase
ALT:	Alanine aminotransferase
ANOVA:	Analysis of variance
BM:	Body mass
BUN:	Blood urea nitrogen
DM	Dry matter
FEN	Fenofibrate
FFAs:	Free fatty acids
FT	*Ficus thonningii* extract
FS	Fructose solution
GIT:	Gastrointestinal tract
HDL-C:	High density lipoprotein cholesterol
HFD	High fructose diet
HFCS	High fructose corn syrup
HDMEFT	High dose methanolic *Ficus thonningii* extract
HOMA-IR:	Homeostatic model of insulin resistance
IDF:	International Diabetes Federation
IR	Insulin resistance
LDL-C:	Low density lipoprotein cholesterol
LI:	Large intestines
LDMEFT	Low dose methanolic *Ficus thonningii* extract
MetS	Metabolic syndrome
MD	Metabolic derangements
NEFAs	Non esterified fatty acids
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
OGTT:	Oral glucose tolerance test
PC:	Plain gelatine cube
PW:	Plain drinking water
PPAR:	Peroxisome proliferators activated receptor
SI:	Small intestine
SD	Sprague Dawley

SSA	Sub-Saharan Africa
T2DM	Type II Diabetes mellitus
TGs	Triglycerides
ß:	Beta
%TBM:	Percentage terminal body mass
rTL:	Relative to tibia length
VLDL:	Very low density lipoprotein
WC	Waist circumference
w/v:	weight/volume
WHO:	World Health Organization

CHAPTER 1: INTRODUCTION

1.0 Preview of the dissertation

This dissertation consists of seven chapters: the introduction, literature review, materials and methods, results, discussion, conclusion and recommendations and references.

Chapter one: is an introductory chapter which highlights the problem of obesity as one of the major drivers of metabolic derangements such as metabolic syndrome (MetS) in growing children. The limitations associated with the use of conventional pharmacological agents in the management of diet-induced obesity and the associated metabolic derangements are highlighted in this chapter. Importantly, a discourse on the need to evaluate the efficacy of plant-derived ethnomedicines to protect against diet-induced metabolic derangements in growing rat models is given. The chapter concludes by stating the aim, objectives and hypothesis of the study.

Chapter two: provides a critical review of the literature pertinent to the study. It gives relevant statistics of the problem of obesity and metabolic abnormalities such as type II diabetes and the metabolic syndrome in growing children. This chapter points out the major causes of obesity and associated metabolic derangements in growing children, such as poor dietary habits typified by the consumption of diets rich in saturated fats and sweeteners such as fructose. The shortfalls on the models that have been used to study diet-induced metabolic derangements are highlighted. Animal models and the utilisation of plant-derived ethnomedicines are described focusing on their (plant-derived ethnomedicines) potential protective effects against diet-induced metabolic derangements and the need to establish their safety. The chapter goes on to discuss *Ficus thonningii* as one of the ethnomedicines commonly used in Sub-Saharan Africa with the potential to prevent the diet-induced metabolic derangements due to its health beneficial biological activities, including among others, hypoglycaemic, hypolipidaemic and anti-oxidant activities.

Chapter three: gives a detailed description of the materials used in carrying out the study as well as the methods that were used for the various assays and analyses on the tissues and samples collected. The chapter also describes how parametric and non-parametric data was analysed.

Chapter four: presents the study findings. The results are presented in graphic and tabular form. The effects of the high-fructose diet, fenofibrate and *Ficus thonningii* on the various parameters that were determined/measured during the trial are narrated.

Chapter five: this chapter mainly interprets and discusses the findings of the study and gives meaning to the results by comparing findings of the current study to other relevant studies. The chapter also attempts to explain the possible mechanisms through which the crude methnaolic *Ficus thonningii* stem-bark extract and fenofibrate exerted health beneficial effects and or failed to exert such effects.

Chapter six: states the main conclusion(s) drawn from the study highlights some major drawbacks of the study and makes some recommendations concerning future studies.

Chapter seven: is a list of all references that were cited in the dissertation.

1.1 Introduction

The World Health Organisation (WHO) reported that 13% of the global adult population are obese (WHO, 2018). There is a tremendous increase in childhood obesity with 340 million children obese globally (WHO, 2018). In sub-Saharan Africa (SSA), 10.6% of children are obese (Muthuri et al., 2014) while in South Africa 13% of the children are obese (Pienaar, 2015). In addition to the epigenetic effects, sedentary lifestyles especially in the expanding urban settlements and poor dietary habits typified by the intake of high calorie (high-fat high-fructose) diets are the major causes of obesity (Padwal, 2014; Sekgala et al., 2018). Obesity increases risk of development of metabolic derangements such as dyslipidaemia (Yi Zhang et al., 2014) and metabolic diseases including among others type II diabetes (T2DM), non-alcoholic fatty liver disease (Jensen et al., 2018) and MetS (Matsuzawa, 2014; Tamborlane et al., 2004).

There are many conventional pharmacological agents used in the management of metabolic diseases. Metformin, for instance, is used to manage T2DM and fenofibrate is used to manage dyslipidaemia among MetS patients (Kraja et al., 2010; X. M. Li et al., 2011). However, the use of these conventional pharmaceutical drugs is limited due to high cost, poor accessibility and the side effects that they elicit (Rodgers et al., 2012). In both the developed and developing world, the challenges associated with the use of such conventional medicines has led to a dependence on plant-derived ethnomedicines (Amiot et al., 2016). The widespread global use of medicinal plants is in tandem with the recommendations by the World Health Organisation that advocates for research on and use of plant-derived medicines in order to ensure increased coverage and access to global primary health care (Qi, 2015).

Approximately 80% of the total population of people in SSA (WHO, 2018) and about 27 million people in South Africa depend on plant-derived ethnomedicines for their primary health care (Oyebode et al., 2016). *Ficus thoninngii* a commonly used medicinal plant is used for the management of an array of diseases in sub-Saharan Africa. Traditionally, the infusions and decoctions of its stem-bark have been used to treat sore throat, cold, fever and diarrhoea (Teklehaymanot & Giday, 2007).

The plant (*F. thonningii*) has a host of phytochemicals, including among others, tannins, saponins and flavonoids (Egharevba et al., 2015; Usman H & Usman, 2009). These phytochemicals have a host of bio-beneficial activities among others anti-oxidant, anti-diabetic and anti-obesity (Fitrilia et al., 2015). The anti-oxidant, anti-diabetic and anti-obesity activities of crude *F. thonningii* extracts make it a potential medicinal plant for the prevention of diet-induced metabolic derangements such as obesity, type II diabetes mellitus and dyslipidaemia.

1.2 Justification of study

Previous research on metabolic diseases largely focused on drug-induced metabolic derangements and or diseases, for example streptozotocin-induced (Gondwe, 2007; Pessoa et al., 2015) and alloxan-induced diabetic rat models (Musabayane et al., 2007). The use of chemically-induced models of metabolic derangements and disease, for example the use of streptozotocin and alloxan, fail to accurately mimic real-life diet-induced development of obesity and metabolic derangements in growing children. The majority of these previous studies largely made use of adult animal models (Shahraki et al., 2011), particularly male rats (Shahraki et al., 2011). Recent studies have demonstrated the existence of fundamental differences in the regulation of homeostasis between males and females (Bocarsly et al., 2010). Furthermore, it has also been reported that age is a critical factor in disease progression (De Lima et al., 2013). These sex- and age-linked differences influence the development of obesity and other health outcomes associated with metabolic diseases including MetS (Zore et al., 2018). As such there is a dire need to prioritise research that teases out sex-related and age-specific metabolic differences.

In vivo research in adult rat models has reported the efficacy of *F. thonningii* extracts in the treatment of obesity and management of MetS (Musabayane et al., 2007). Adult murine models, however, do not suitably mimic diet-induced obesity and its associated metabolic disorders and diseases such as MetS in growing children. Since obesity and the MetS are

increasing in children and adolescents (Sekokotla et al., 2017), it is pertinent that animal models that appropriately mimic these metabolic conditions in growing children be utilised in the evaluation of the potential prophylactic effect of medicinal plants. Several conventional pharmacological agents can be used to manage these diet-induced metabolic derangements and diseases. These pharmacological agents include glucose lowering and appetite supressing formulations such as metformin and resveratrol, respectively (Szkudelska & Szkudelski, 2010; Timmers et al., 2011). In addition, some of the formulations also include insulin sensitizers and fenofibrate, a lipid-lowering agent (Damci et al., 2003). However, it has to be noted that each of the conventional pharmacological agents are monotherapeutic and highly specific in their effect and as such fail to be effective against the multiple effects that characterise metabolic derangements and diseases. Medicinal plants such as *Ficus thonningii* contain many biologically active phytochemical compounds that exert health benefits through their multiple mechanisms of action (Egharevba et al., 2015).

1.3 Aim of study

The study sought to investigate the efficacy of crude aqueous *F. thonningii* stem-bark extracts to protect against the development of diet-induced metabolic derangements in growing male and female Sprague Dawley rats fed a high-fructose diet mimicking growing children fed obesogenic diets.

1.4 Objectives

The specific objectives of the study were to:

a. Determine the phytochemical composition of crude methanolic *F. thonningii* stem-bark extracts.
b. Determine, in high-fructose diet fed growing male and female Sprague Dawley rats, the effects of crude methanolic *F. thonningii* stem-bark extract on:

i. growth performance: using body mass and long bone-based indices.
ii. the ability to tolerate a glucose load.
iii. viscera organ macro- and micro-morphometry.
iv. plasma concentration of circulating metabolic substrate (glucose, cholesterol and triglycerides) concentration as well as hepatic (stored metabolic substrate) lipid content.
v. red blood cell osmotic fragility.
vi. liver lipid storage
vii. hepatic function by determining plasma activities of alanine aminotransferase (ALT) and alkaline phosphatase (ALP) and histological analyses

viii. kidney function by determining surrogate markers (plasma creatinine, and blood urea nitrogen) of kidney function and histological analyses

ix. insulin resistance (IR) and β-cell function by measuring plasma insulin concentration and computing HOMA-IR index.

1.5 Hypothesis

The hypotheses of the study were:

H_0: Crude methanolic *F. thonningii* stem-bark extracts do not affect the growth performance, tolerance of an oral glucose load, viscera macro- and micro-morphometry and the health of growing male and female Sprague Dawley rats fed a high-fructose diet.

H_1: Crude methanolic *F. thonningii* stem-bark extracts affect the growth performance, tolerance of an oral glucose load, GIT macro- and micro-morphometry and the health of growing male and female Sprague Dawley rats fed a high-fructose diet.

CHAPTER 2: LITERATURE REVIEW

2.1 Obesity

Obesity is defined as the excessive accumulation of fat in the body that impairs health (WHO, 2014). Body mass index (BMI) has historically been the gold standard in anthropometric measurement of obesity (Clark et al., 2016), with a BMI of greater than 30kg/m^2 indicating obesity in adults (WHO, 2014). However, BMI is not considered a reliable index for quantifying obesity in children and adolescents as they undergo several physiological changes in body shape as they grow. In addition, BMI does not accurately represent body fat distribution as it fails to distinguish between fat and fat-free mass (Adab et al., 2018). Direct measurement of "fatness" such as densitometry and bioelectrical impedance analysis (BIA) are ideal methods of determining obesity in children (Al-sindi, 2014). These methods, are however, time consuming, expensive and as such may not be used where research in a large population are involved (Ross et al., 1993). Waist circumference is an accurate determinant of obesity in children and adults as it is correlated to visceral adiposity (Adab et al., 2018).

Visceral or central obesity is defined as the abnormal high deposition of adipose tissue which increases the risk factor for developing T2DM, hypertension and atherosclerosis (Sandeep et al., 2010). Visceral adiposity is one of the major risk factors for the development of IR and dyslipidaemia in children (Gastaldelli et al., 2007). Adipose tissue secretes a large number of cytokines and other metabolites, which are bioactive (Fasshauer & Blüher, 2015). Cytokines, which are cell-signalling molecules, stimulate and promote inflammation and IR (Kwon & Pessin, 2013). In addition to the adipose-tissue induced inflammation and IR, research has shown that increased visceral adiposity and elicits lipolysis resulting in a greater flux of free fatty acids (FFA) into the portal circulation (Ebbert & Jensen, 2013). The oversupply of FFA to the liver results in impaired metabolic pathways resulting in altered glucose and lipid metabolism (Mansour, 2014).

Obesity is caused by multiple factors ranging from the genetic predisposition to environmental factors such as poor dietary habits and sedentary lifestyles (Anderson et al., 2018). While genetic factors have been shown to account for less than 5% of cases of childhood obesity (Anderson et al., 2018) they still significantly contribute to the observed increase in the prevalence of obesity. Recent studies suggests that excessive consumption of diets high in fructose to be linked to weight gain and the increase in obesity and diet-induced metabolic disorders particularly in children and adolescents (Bray, 2010; Kania, 2016)

2.2 Fructose consumption and the development of metabolic derangements

Fructose is a 6-carbon monosaccharide naturally found in vegetables, fruits, and honey (Yong Zhang et al., 2014). It is commercially manufactured from corn as high fructose corn syrup (HFCS) which is both palatable and affordable. The HFCS is used to sweeten foodstuffs and and beverages which children and adolescents tend to consume more than healthier alternatives (Maarman et al., 2016). Following ingestion, fructose is rapidly absorbed from the jejunum via the fructose-specific glucose transporter (GLUT5) (Rutledge and Adeli, 2007). GLUT5 is expressed in insulin-sensitive tissues (skeletal muscle and adipocytes), where it mediates the uptake of substantial quantities of dietary fructose (Hannou *et al.*, 2018). GLUT2 transports fructose out of the jejunal absorptive enterocytes into the blood where it is then transported to the liver (Stolarczyk, 2005). In the liver, fructokinase then catalyses the phosphorylation of fructose to fructose-1-phosphate (Hannou *et al.*, 2018). The resultant fructose-1-phosphate is then converted into 3-carbon phosphate intermediates: glyceraldehyde-3- phosphate and dihydroxyacetone phosphate via catalysis by aldolase B (Rutledge & Adeli, 2007). These 3-carbon phosphate intermediates are known precursors of triglycerides (TGs) synthesis (Rutledge & Adeli, 2007) as they are utilised for the synthesis of glycerol and fatty acids (Nye et al., 2008) . Thus, the excessive consumption of fructose results in large amounts of substrates that are utilised in the synthesis of TGs; a situation exacerbated by the relative lack of regulation of this TGs synthetic pathway (Basciano et al., 2005b). The TGs can be packaged into very-low density lipoproteins (VLDLs) by the liver. As the VLDLs are transported through the bloodstream, lipoprotein lipase hydrolyses them into TGs generating non esterified fatty acids (NEFAs) and monoacylglycerol that on being taken up by adipose tissue are reconstituted into TGs (Khitan & Kim, 2013). Therefore, the excessive consumption of fructose can lead to high levels of circulating free fatty acids and increased adiposity (obesity) and associated metabolic derangements. These metabolic derangements increase the risk of the development of metabolic diseases among many T2DM, IR, hypertension, dyslipidaemia, NAFLD and coronary heart disease (Angelopoulos et al., 2009; Ang and Yu, 2018). This cluster of obesity-associated metabolic abnormalities is collectively termed as the metabolic syndrome.

2.2.1 Metabolic syndrome

Metabolic syndrome refers to the manifestation of a collection of risk factors that increase the risk of developing T2DM and cardiovascular diseases (Grundy, 2016; Neill & Driscoll,

2015). These risk factors include visceral adiposity (obesity), insulin resistance, hyperglycaemia, hypertriglyceridaemia, increased low density lipoprotein (LDL), decreased high density lipoprotein (HDL) and hypertension (Kania, 2016). According to the International Diabetes Federation guidelines (IDF) and the WHO guidelines, metabolic syndrome in children and adolescents is diagnosed when patients present with central obesity with a waist circumference of >90th percentile and any other two of hyperglycaemia (fasting blood glucose of >5.6mmol/L), hypertriglyceridaemia (TGs ≥1.69mmol/L), high blood pressure (BP of >130/85mmHg) and decreased high density lipoprotein (HDL <1.04 mmol/L) (Grundy, 2016). In Africa, MetS amongst children and adolescents is on the increase with a prevalence of between 10% and 50% depending on setting of the studied population (Fezeu et al., 2007). The current trend of increase in the prevalence of the metabolic syndrome is largely and generally attributed to the higher consumption of dietary fructose (Toop & Gentili, 2016)

2.2.2 Insulin resistance and hyperglycaemia

Insulin resistance (IR) is a pathological condition in which physiological insulin concentration does not elicit a normal response in the liver, skeletal muscle and adipose tissue (Kaur, 2014). Insulin promotes substrate storage in the liver, fat and muscle by stimulating glycogen synthesis, lipogenesis and lipid storage while inhibiting lipolysis and glycogenolysis (Basciano et al., 2005a). An IR-induced failure to stimulate these metabolic actions results in elevated fasting glucose and triglyceride levels (Burgeiro et al., 2017). Indirect methods for quantifying diet-induced insulin resistance such as the oral glucose tolerance test (OGTT) and the homeostasis model assessment of insulin resistance (HOMA-IR) have been used in several studies (Singh *et al.*, 2013). A HOMA-IR value of 2.5 is taken as an indicator of insulin resistance in children and adolescents (Singh *et al.*, 2013). Several studies in humans and rodents have shown that moderate to high consumption of fructose leads to an increase in insulin resistance (Gupta et al., 2014; Suwannaphet et al., 2010). Under normal physiological conditions, insulin suppresses lipolysis in adipocytes, thus an impaired insulin signalling increases lipolysis, resulting in increased NEFAs levels leading to the dyslipidaemia which manifests as hypertriglyceridaemia. Therefore, insulin resistance results in hyperglycaemia which leads to dyslipidaemia (Gall *et al.*, 2013).

2.2.3 Dyslipidaemia

Diet–induced dyslipidaemia manifest as an abnormal amount of lipids in the blood which is characterised by the high concentrations of plasma TGs, cholesterol and low density lipoproteins (Ginsberg *et al* 2006). Previous studies in humans and rodents have shown that the consumption of high fructose diets leads to an increase in *de novo* lipogenesis and circulating TGs (Crescenzo et al., 2012; Stanhope & Havel, 2008). A plasma triglyceride concentration level of >1.7mmol/L and or HDL-cholesterol concentration of <0.9 mmol/L in males and or <1.0mmol/L in females is diagnostic of dyslipidaemia (Nielsen et al., 2017). In the liver, FFAs serve as a substrate for the synthesis of TGs. Additionally FFAs also stabilise the production of apolipoprotein B (apoB) the major lipoprotein of very low density lipoprotein (VLDL) particles, resulting in a more VLDL production (Yuan et al., 2007). Thus insulin resistance is the result of both an increase in VLDL production and a decrease in VLDL clearance (Holzl et al., 1998) .

In addition to dyslipidaemia, unhealthy diets also cause non-alcoholic fatty liver disease.

2.2.4 Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is defined as the deposition of TGs in the liver which is >5% of the total liver weight in the absence of alcohol consumption or any underlying steatogenic medical condition or drugs (Lee et al., 2010). The first stage of NAFLD is characterised by the accumulation of lipids in the liver (steatosis) which is considered as the hallmark of NAFLD (Michelotti et al., 2013). Steatosis may progress to non-alcoholic steatohepatitis (NASH) which is characterised by hepatocyte damage and inflammation (Hassan et al., 2014). NAFLD is the most common liver diseases affecting 25% of adults and 8% of children globally (Mohan et al.,, 2019). Its prevalence (NAFLD) is said to increase with that of T2DM, obesity and MetS (Younossi et al., 2017). Among the several factors contributing to the development of NAFLD, the consumption of a high-fructose diet is a major contributor (Jegatheesan & De Bandt, 2017). Its consumption has been shown to induce the disease in rodents and humans (Alwahsh & Gebhardt, 2017) Dietary fructose-induced NAFLD involves several pathological processes among them lipogenesis (Lim et al., 2010). The latter leads to mitochondrial dysfunction (Vos & Lavine, 2013) and stimulates activation of inflammatory pathways leading to the development of IR (Lim et al., 2010).

2.2.5 Diabetes mellitus

The American Diabetes Association (ADA) defines diabetes mellitus as ‘a group of metabolic diseases characterised by hyperglycaemia resulting from defects in insulin secretion, insulin action or both’ (American Diabetes Association, 2014). Globally, diabetes mellitus is now considered the most common non-communicable disease and the fourth leading cause of death in developed countries (WHO, 2018). In 2017, an estimated 451 million adults had diabetes mellitus worldwide (WHO, 2018). In SSA, an estimated 15.5 million adults had diabetes mellitus in the year 2017 (Karuranga et al., 2018) and the disease was estimated increase 162.5% fold by 2045 (Karuranga et al., 2018). There are two types of diabetes mellitus: type I and type II diabetes mellitus. The former occurs due to autoimmune destruction of endocrine pancreatic β-cells (American Diabetes Association, 2014) while the latter results from either altered insulin secretion and or IR (Zaccardi et al., 2016). Type II diabetes mellitus the most common form of the two, accounts for approximately 90% of all diabetic patients globally (Wild *et al.*, 2004). It is well established that obesity is the main aetiological cause of T2DM, hence the adoption of the term ‘diabesity” (Astrup & Finer, 2000) to describe the increasing incidence of diabetes in combination with obesity. To further study obesity and related metabolic diseases such as diabetes mellitus, several animal models have been utilised.

2.3 Animal models of obesity-related metabolic derrangements

Several experimental models mimic aspects of metabolic diseases. These models are utilised in the evaluation of potential therapeutic interventions. Some of the models currently in used include dietary, genetic and pharmacological rat models.

2.3.1 Chemically-induced models

Pharmacologically-induced models of metabolic derangements are largely exemplified by streptozotozin or alloxan (Wong et al., 2016). Alloxan and streptozotocin are toxic glucose analogues that preferentially accumulate in pancreatic beta cells via the GLUT2 glucose transporter (Lenzen, 2008). These agents (streptozotocin and alloxan) selectively destroy the endocrine pancreatic β-cells, thus disrupting their insulin secretory capacity (Brown & Panchal, 2011). High doses of alloxan induced type I diabetes mellitus, whilst low doses are suited for T2DM (Liu et al., 2010). However, both models do not sufficiently mimic the metabolic syndrome. In an effort to develop a better model, researchers have used a combination of streptozotocin at low doses in a nutritional model (high fat or high fructose) of

the MetS (M. S. Islam & Wilson, 2012). The chemically-induced models do not accurately and practically mimic real life situations where unhealthy diets can triggers metabolic derangements.

2.3.2 Genetic models

Genetic animal models are imperative in order to investigate the pathogenesis of metabolic derangements that are influenced by genetic factors. Most genetic models that have been established are based on monogenic mutations ((Bertram & Hanson, 2001). These genetic models include leptin-deficient (*ob/ob*) mice, leptin receptor-deficient (*db/db*) mice, Zucker fatty (ZF) rats, Zucker diabetic fatty (ZDF) rats, Goto-Kakizaki (GK) rats and the Otsuka Long-Evans Tokushima Fatty rats (Brown & Panchal, 2011; Wong et al., 2016). These models are useful in evaluating specific molecular mechanisms that may be involved in the development of obesity and obesity-associated metabolic dysfunctions in rodents. However, in humans, the metabolic dysfunctions is not a monogenetic disorder hence these models do not show the range of signs that characterise the full range of signs and symptoms associated with metabolic dysfunctions of the nature of MetS. Newer models that are polygenic such as the Zucker diabetic Sprague dawley rats (ZDSD) have been developed (Peterson *et al.,* 2015). However they have a major drawback of not being relevant to the current etiology of metabolic derangements in humans which is largely dietary in origin (Rutledge and Adeli, 2007).

2.3.3 Diet-induced models

Over-nutrition has been considered as one of the key contributors to the incidence of obesity and associated metabolic derangements inclusive of the metabolic syndrome. Diet-induced animal models therefore represent, a predominantly, environmentally acquired obesity and its associated metabolic derangements and diseases (Oron-Herman et al., 2008). However, lack of standardised methodology for induction of metabolic derangements has resulted in a variety of high-calorie diets being used each with different nutritional values, formulations and preparation methods (Barrett et al., 2016). The most common diets used are those high in fructose, sucrose, saturated fat or a combination of these macronutrients (Parasuraman & Wen, 2015) . However, it has since been established that dietary fructose promotes *de novo* lipogenesis which causes a deranged plasma lipid profile, ectopic lipid deposition, which in turn causes peripheral IR and progressive organ damage in humans and rodents (Lim et al., 2010; Oron-Herman et al., 2008). High-fructose feeding to rodents induced the development

of symptoms of MetS and other metabolic diseases seen in humans including IR, impaired glucose tolerance and dyslipidaemia (Tappy & Rosset, 2017). Consequently, a high-fructose diet model has been used for investigating the development of diet-induced MetS and related metabolic derangements. Different amounts of fructose and modes of intake have been utilised which have resulted in the expression of a range of features of diet-induced metabolic diseases (Brown & Panchal, 2011). This is done by manipulating the quantities and duration of fructose feeding in order to mimic the development of diet-induced obesity and associated metabolic derangements in humans, hence the continued use of the high-fructose diet model in research (Sa´nchez-Lozada, *et al* 2007).

Conventional therapies such as fenofibrate and metformin are used to treat diet-induced metabolic disorders. Some of these conventional therapeutic pharmacological agents used to treat diet-induced metabolic derangements include statins which are blood lipid lowering agents and metformin which lowers blood glucose concentration (Damci et al., 2003; Pinteur et al., 2007). In the current study, I used fenofibrate as a positive control pharmacological agent for comparison with crude methanolic *F. thonningii* stem bark extracts. The section that follows describes the mechanisms by which fenofibrate exerts its effects.

2.4 Fenofibrate: mechanism of action

The lipid-modifying effects of fenofibrate are mediated through the activation of peroxisome proliferator receptor alpha (PPAR-α) (Packard, 1998). PPAR-α is a ligand-activated transcription factor which regulates the expression of genes involved in β-oxidation of fatty acids (Packard, 1998). Activated PPAR-α, increases lipolysis and eliminates plasma TGs by upregulating the synthesis of proteins responsible for fatty acid transport and β-oxidation, which inhibits the formation of TGs and VLDL (Staels et al., 1998). Triglyceride levels are further reduced due to upregulation of the synthesis of lipoprotein lipase and apolipoprotein (Apo)-V and downregulation of Apo-CIII (Noonan et al., 2013). A consequence of these physico-biochemical changes is a shift in the balance of LDL species from small, dense particles toward larger, more buoyant particles that are more easily cleared by the LDL receptor and less likely to become oxidised (Keating & McKeage, 2012).

The presence of phytochemicals with health beneficial biological activities is central to the use of plant extract to manage disease. *Ficus thonningii* is one of the commonly used medicinal plants in ethnomedicine.

2.5 *Ficus thonningii*

2.5.1 Botanical description

Ficus thonningii, family *Moraceae*, order, *Rosales* (Burrows and Burrows, 2003; Coates and Palgrave, 2013) is a deciduous tree which grows up to 21m in height and has a rounded to spreading and dense canopy (Orwa et al., 2009). The tree which is widely distributed is largely found at altitudes of 1000-2500m and in areas within a minimum rainfall of 800mm (Orwa et al., 2009). Its stem bark on young branches is hairy, but smooth and grey on older branches. The whole plant exudes milky latex often turning pinkish. The leaves are dark, thin and papery or slightly leathery, with smooth, obovate or elliptic margins though sometimes elongated or slightly oblanceolate (Yusuf & Muritala, 2013). Figs are found in leaf axils or below the leaves enclosing many small flowers mostly hairy and borne in the leaf axils (Orwa et al., 2009). Its flowers are pollinated by wasps and the seeds are dispersed by bats (Orwa et al., 2009) . In Southern Africa, flowering and fruiting are observed for most of the year with the peak period in October (Orwa et al., 2009).

2.5.2 Dietary uses

The leaves are a good source of proteins as well as calcium and zinc (Otitoju et al., 2014). Owing to their high nutritional content *F. thonningii* leaves are utilised as a vegetable by Nigerians and the Senegalese especially during the drought season when the main food sources are generally scarce (Diouf et al., 2007; Igoli et al., 2016). In addition to the high protein and calcium and zinc content, *F. thonningii* leaves and succulent stems have a high fibre content hence their use as supplementary fodder during periods of feed scarcity (Jokthan et al., 2009). The ripe fruits of *F. thonningii* are a rich source of carbohydrates and essential fatty amino acids and essential fatty acids (Igoli et al., 2016).

2.5.3 Ethnomedicinal uses of *F. thonningii*

2.5.3.1 Stem bark

In West Africa a decoction of the stem bark of *F. thonningii* is used to treat cough and throat infections (Igoli et al., 2016). Traditionally, the stem bark extract of *F. thonningii* has been used for the treatment of arthritis, diarrhoea, ulcers (Teklehaymanot & Giday, 2007) and to boost fertility (Obata & Aigbokhan, 2012). Stem-bark extracts are used in baths to treat

nervous illness, tuberculosis, paralysis and leprosy (Badiora et al., 2016) In Ethiopia, the inner bark is chewed to relieve stomach-ache while the powdered bark is used on wounds (Teklehaymanot & Giday, 2007). Stem bark extracts have also been reportedly used to enhance fertility (Shomkegh et al., 2016), induce the menstrual cycle, stimulate lactation and aid parturition (Orwa et al., 2009). Other medicinal uses of the stem bark reported include treatment of dysentery, constipation, and nose bleeding (Badiora et al., 2016).

2.5.3.2 Leaves

F. thonningii leaves are traditionally used to treat diarrhoea, gonorrhoea, diabetes mellitus (Orwa et al., 2009) and leaf extracts are used to treat bronchitis, bone movement disorders, urinary tract infections, thrush, scabies (Igoli et al., 2016) . In Nigeria, *F. thonningii* leaf decoction is taken orally to improve appetite and to treat chicken pox when topically applied (Oladunmoye et al., 2016).

2.5.3.3 Latex

F. thonningii exudes white sticky latex that turns pinkish with time. The latex is dropped in the eye to treat cataracts (Alawa et al., 2002). Traditionally, the latex is used as a galactogue and vermifuge (Orwa et al., 2009) as well as to treat fever, tooth decay and ringworm (Alawa et al., 2002).

2.5.3.4 Roots

Roots of the plant have been reportedly used for preventing miscarriages and for stopping nose-bleeding (Alawa et al., 2002). Additionally, the roots are also used for relieving stomach pains and diarrhoea (Teklehaymanot & Giday, 2007), pneumonia and chest pains. In Mpumalanga, South Africa, an infusion of the root is used to treat eye problems (Tshikalange et al., 2016).

2.5.4 Phytochemical composition

The medicinal properties of the *F. thonningii* extracts have been attributed to an array of its phytochemical constituents. There has been little quantitative analysis of *F. thonningii* extracts that has been done. Most researchers have used qualitative methods to determine the presence or absence of a class of phytochemicals in *F. thonningii*. Qualitative phytochemical analysis of *F. thonningii* leaves and stem-bark have revealed the presence of many phytochemicals, including among others, tannins, saponins, flavonoids, anthraquinones,

carbohydrates and alkaloids (Ndukwe et al., 2007; Coker et al., 2017). Ndukwe et al. (2007) has reported the presence of flavonoids in the stem-bark, roots and leaves of *F. thonningii*. Two new flavonoids namely, thonningiol and thonningiisoflavone have also been isolated from the stem-bark and fruits of *F. thonningii* (Ali et al., 2014). The phytochemicals in *F. thonningii* have anti-oxidative, anti-diabetic and anti-lipidaemic properties (Marrelli et al., 2016).

2.5.4.1 Alkaloids

Alkaloids are a large class of plant secondary metabolites comprised of low molecular weight, nitrogen-containing compounds (Richard et al., 2013). They are biologically significant as stimulators, inhibitors and terminators of growth (Swarnalatha et al., 2018). They are distributed in different parts of the plant for example, nicotine in leaves, cinchonine and quinine in bark, strychnine and nibidine in seeds and rawelfinine and glycyrrhizin in roots (Kuete, 2013). *F. thonningii* contains alkaloids (Ndukwe et al., 2007; Victor Masekaven & Adenkola, 2013) but literature on specific alkaloids isolated from *F. thonningii* is scanty.

2.5.4.2 Tannins

These polyphenolic compounds, some hydrolysable and other insoluble in water, are present in many plant foods (Chung et al., 1998). They have anti-oxidant, hypoglycaemic and hypolipidaemic properties (Hagerman et al., 1998). Tannin concentrations in *F. thonningii* leaves has been estimated to be about 90mgL/100mg dry matter (Bamikole et al., 2004; Yusuf & Muritala, 2013).

2.5.4.2 Saponins

The concentration of saponins in *F. thonningii* leaves was shown to be as high as 300 mg/100 g DM (Bamikole et al., 2004). Apart from being associated with reduced feed intake, retarded growth and lysis of human erythrocytes (Kumar, 1992), saponin-containing plants are toxic to ruminants wherein they cause liver and kidney degeneration (Wina et al., 2005). Besides these negative effects, saponins also exhibit health beneficial pharmacological properties including, among others, hypolipidaemic and hypoglycaemic (Netala et al., 2015).

2.5.5 Biological activities

2.5.5.1 Anti-oxidant properties

Studies have reported that crude methanolic and aqueous *F. thonningii* stem-bark extracts have stronger antioxidant activity than that of leaves and fruits (Sirisha et al., 2010). The antioxidant activity is mainly due to the presence of the isolated flavonoids orientin, vitexin and isovitexin which possess antioxidant properties and free radical scavenging activity (Grassi et al., 2010). In a study conducted by Gaire and colleagues (2017), the antioxidant potential of crude methanolic *F. thonningii* stem bark extract was comparable to that of tannic acid.

2.5.5.2 Cardioprotective effects

Ficus thonningii possesses cardio-suppressant and hypotensive properties (Musabayane, 2012). In a study by Musabayane and collegues (2007), ethanolic stem bark extracts of *F. thonningii* were shown to exhibit positive chronotropic and inotropic effects on both electronically driven and spontaneously beating atrial muscle strips. Results of the same study also showed an attenuating effect of *F. thonningii* extract (120 mg/kg b.m. given for 5 weeks) on mean arterial pressures.

2.5.5.3 Hypoglycaemic effects

F. thonningii has been shown to exhibit hypoglycaemic effects in rats (Musabayane, 2012). Oral glucose tolerance tests performed on streptozotocin-iduced diabetic and non-diabetic rats treated with ethanolic *F. thonningii* stem bark extract showed a dose dependant hypoglycaemic effect comparable to that of metformin; the positive control (Gondwe, 2007) . Similar findings were also reported where methanolic *F. thonningii* leaf extracts lowered circulating glucose concentration in hypercholesteromic rats (Ahur et al., 2014).

2.5.5.4 Hypolipidaemic effects

F. thonningii has been reported to possess hypolipidaemic properties. *F. thonningii* methanolic leaf extracts significantly lowered serum triglycerides, total cholesterol, low density lipoprotein (LDL), very low density lipoprotein (VLDL) but enhanced high density lipoprotein (HDL) values in hypercholesterolemic rats (Ahur et al., 2014). The hypolipidaemic properties of *F. thonningii* could be attributed to the presence of flavonoids

which have been shown to possess hypolipidaemic effects by preventing LDL-oxidation, a prerequisite for hyperlipidaemia (Grassi et al., 2010).

2.5.5.6 Haematinic effects

Ethanolic *F. thonningii* leaf extracts exhibit haematinic properties. Ahur et al. (2013) reported an improvement in haematological parameters such as haematocrit and red blood cell count in acetaminophen-induced rats. Dangarembizi et al. (2014) reported the same findings where methanolic *F.thonningii* leaf extracts improved packed cell volume (PCV) levels in high-fructose fed growing Sprague Dawley rats. This validates the traditional use of *F.thonningii* in the treatment of anaemia.

CHAPTER 3: MATERIALS AND METHODS

3.1 Plant collection and identification

Fresh *F. thonningii* stem bark was collected in July 2016 at a farm in Bulawayo, Matebeleland North province, Zimbabwe. The farm is on longitude 20° 13' 47" and latitude 28° 45' 9". The area has red, loam type soil that receives an average annual rainfall of 550mm and has minimum and maximum temperatures of 14.9°C and 28.1°C (Sibanda et al., 2018), respectively. The fresh stem bark and samples of the trees' small branches were transported overnight to Johannesburg, University of the Witwatersrand. A sample was sent to a nature conservationist Mr John Burrows for identification and authentication.

3.2 Extract preparation and storage

The stem bark of *F. thonningii* were cut into thin strips and dried in an oven (Salvis ®, Salvis Lab, Switzerland) at 40°C for 24hours. The dried strips were milled into a fine powder using a blender (Russel Hobbs, England). The crude extract of *F. thonningii* was prepared by macerating every 25g of dry bark powder in 100mL of 80% methanol (Merck Chemicals, Johannesburg South Africa) for 24 hours (Musabayane et al., 2007). During the 24-hour maceration, the mixture was continuously stirred. Immediately after the maceration, the mixture was filtered using a Whatman No. 1 filter paper (Whatman®, No 1, size 185mm, pore size 7-11 Town/City England). The residue was discarded and the filtrate was concentrated by evaporating it at 60°C in a rotor evaporator [Labocon (Pty) Ltd, Krugersdorp, South Africa] and then dried in an oven (Salvis ®, Salvis Lab, Switzerland) at 40°C for 12 hours (Okwari and Ofemi, 2011). The dried extract was recovered, weighed and kept in a tightly sealed glass bottle at 4°C until use.

3.3 Determination of phytochemical content

In order to determine the presence or absence of major classes of phytochemicals in *F.thonningii* stem bark-extract, qualitative screening tests were carried out.

3.3.1 Test for tepernoids

The test for terpenoids was done as described by Ayoola et al. (2008) Briefly, 0.5g of the *F.thonningii* stem-bark extract was added to 2mL of chloroform in a test tube after which 3mL of concentrated sulphuric acid was added to the mixture. The development of a brown reddish colour in the interface layer indicated the presence of terpenoids.

3.3.2 Test for saponins

The presence of saponins was determined as described by Ayoola et al. (2008) . Briefly, 0.5g of crude methanolic stem bark extract was added to 5mL of distilled water in a test tube and shaken vigorously for 30 seconds. Persistent frothing confirmed the presence of saponins.

3.3.3 Test for flavonoids

The test for flavonoids was determined as described by Siddique et al. (2013). Briefly 0.5g of crude methanolic *F. thonningii* stem-bark extract was dissolved in 50mL of methanol. 2mL of the crude methanolic *F. thonningii* stem-bark extract was added to 5mL of 10% ammonium hydroxide. The mixture was thoroughly mixed after which 1 mL of concentrated sulphuric acid was added. A yellow colour indicated the presence of flavonoids.

3.3.4 Test for tannins

The test for tannins in *F.thonningii* stem-bark extract was determined as described by Ayoola et al. (2008). Briefly, 0.5mg of the crude *F.thonningii* stem-bark extract was boiled in 5mL of distilled water following which three drops of ferric chloride was added. A dark purple black colour indicated the presence of tannins.

3.4 Animal ethical clearance and study site

The use of animal in this study was approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (AESC number: 2016/05/24/C). Animal studies were carried out in the Central Animal Services at the University of the Witwatersrand. The various assays on collected samples were performed in Laboratories of the School of Physiology, University of the Witwatersrand.

3.5 Experimental animals, housing and feeding

A total of eighty 21-day old Sprague Dawley rat pups were used. The rat pups were allowed a 2-day habituation period before commencement of the experiment. Each rat was individually housed in an acrylic cage wherein wood shavings were used for bedding and were changed twice a week. Room temperature was maintained at 24±2°C, and light/dark cycle maintained with lights on between 7am to 7pm. Adequate positive pressure ventilation was maintained at all times. The rats had *ad libitum* access to standard rat chow (Epol®, Centurion, South Africa) and either plain drinking water or 20%(w/v) fructose solution.

3.6 Experimental design

Eighty 23-day old (40 male; 40 female) Sprague Dawley rat pups were randomly allocated to and administered the following treatment regimens: group 1- standard rat chow (SRC) + plain cubes and plain drinking water, group 2- SRC + plain cubes + 20% (w/v) fructose in drinking water, group 3- SRC + 20% (w/v) fructose solution + fenofibrate in gelatine cube at 100 mg/kg b.m daily, group 4- SRC + 20% (w/v) fructose solution + *F. thonningii* extract in gelatine cube at 50 mg/kg b.m daily and group 5- SRC + 20% (w/v) fructose solution + *F. thonningii* extract in gelatine cube at 500 mg/kg b.m daily. The treatment regimens were administered for 8 weeks. The dosages used for *F. thonningii* and fenofibrate used in this study were previously used without adverse effects on health (Muhammad, 2017). The gelatine cubes used as a vehicle for either the fenofibrate or *F. thonningii* extract were prepared as described by Kamerman et al. (2004). The rats were weighed using an electronic balance (Snowrex Electronic Scale, Clover Scales, Johannesburg, South Africa) twice per week to assess growth performance and to maintain a constant dose of the *F. thonningii* stem bark extract and fenofibrate relative to body mass over the 8-week treatment period.

3.7 Oral glucose tolerance test

After 8-weeks of treatment, the rats were subjected to an oral glucose tolerance test (OGTT) following a two-day habituation to restraining in perspex rat restrainers for two hours. On post-natal day 77, the rats were fasted overnight but were allowed *ad libitum* access to drinking water. Fasting blood glucose was then determined using a Contour-plus glucometer (Contour Plus®, Bayer Corporation, and Mishawaka, USA) as per the manufacturer's instructions. Briefly, blood was taken from a pin-prick of the tail vein following sterilisation of the area to be pricked with a cotton swab impregnated with ethanol for disinfection (Loxham et al., 2007). Following the determination of the blood glucose concentration 2g/kg body mass of sterile 50% (w/v) D-glucose solution (Sigma, Johannesburg, South Africa) was then administered to each rat via orogastric gavage (Loxham et al., 2007). Thereafter blood glucose concentrations were measured at 15, 30, 60 and 120 minutes post-gavaged as previously described by Imam et al (2009).

3.8 Terminal Procedures

Following OGTT, the rats were returned to their respective treatment regimens for 48-hours and then fasted for 12-hours before being euthanised by an overdose intraperitoneal injection of sodium pentobarbitone (200mg/kg) body mass; Euthanaze, Centaur labs, Johannesburg,

South Africa). Prior to euthanasia, blood was drawn from a pin prick at the tip of the tail and used to measure fasting glucose and triglyceride concentration using a calibrated glucometer (Contour Plus®, Bayer Corporation, Mishawaka, USA) and a calibrated Accutrend GCT meter (Accutrend, Roche Mannheim, Germany), respectively according to the manufacturers' instructions. An additional drop of blood was used to determine packed cell volume and blood haemoglobin count using an HCT meter (Woodly Equipment Company, Lancashire, UK) according to the manufacturer's instructions. After euthanasia blood sample was collected from each rat via cardiac puncture using 21G needles mounted onto 10 ml syringes. Approximately 6ml of blood were transferred into heparinised blood tubes and the rest into plain tubes. The blood samples were centrifuged for 10 min at 5000 × *g* in a centrifuge (SorvallRT ®6000B, Pegasus Scientific Inc., Rockville USA). The harvested plasma and serum was then put in microtubes and then stored at -20°C pending assays.

3.8.1 Determination of viscera macro-morphometry

Following blood collection, the viscera (heart, liver, stomach small and large intestines, pancreas, caecum, visceral fat, epididymal fat, testes and kidneys) were carefully dissected out and weighed on an electronic balance (Precisa balance 310M, Instrulab, Switzerland). The intestines (small and large) were gently stretched out and their lengths were measured using a ruler mounted on a cooled dissecting board. Residual digesta in the stomach, small and large intestines and caecum was gently squeezed out prior to weighing the organ. Samples of the small intestine, liver and whole kidneys were collected and preserved in 10% phosphate buffered formalin pending histological analyses. The rest of the liver samples were stored at -20°C for determination of hepatic lipid content.

3.8.2 Determination of osmotic fragility

Osmotic fragility of the erythrocytes was determined as described by Baker and Silverton (1980). Briefly, serially diluted phosphate buffered saline stock solutions were prepared and 5mL aliquoted into 13 tubes. Thereafter 50µL of heparinised blood was added into each test tube, mixed gently by inverting the tubes and then incubated at room temperature for 30 min. The test tubes were then centrifuged at 5 000 × *g* for 10 min (Hermle Centrifuge Z230A, Berthold Hermle AG, German). The supernant from each test tube was decanted into cuvettes and the degree of haemolysis measured using a spectrophotometer (Beckman coulter DU ®) at 540nm. The percentage haemolysis (PH) was calculated according to Faulkner and King (1970) using the equation:

$$\text{Percent haemolysis} = \frac{Optical\ density\ of\ supernant\ of\ blood\ in\ PBS}{Optical\ density\ of\ supernant\ from\ blood\ in\ distilled\ water} \times 100$$

3.8 Determination of liver lipid content

The liver lipid content was determined at the South African National Accreditation System accredited laboratory at the Agricultural Research Council (Irene Analytical Services Laboratory) using Tecator Soxtec apparatus as described by the Association of Analytical Chemists (AOAC, 2005; method number 920.39). The assays were done in duplicate.

3.9 Determination of clinical biochemistry

The plasma activities of alanine amino transferase (ALT), alkaline phosphatase (ALP) (surrogate markers of liver function) and concentration of blood urea nitrogen (BUN), creatinine and cholesterol was determined by a calibrated Clinical Chemistry Analyser (IDEXX VetTest®, Clinical Chemistry Analyser, IDEXX Laboratories Inc., USA) following the manufacturer's instructions. Briefly, thawed samples were gently inverted to mix contents, and 150µL were pipetted into a sample cup which was placed into the analyser. Ten microliters of plasma were dispensed onto each pre-loaded disc in succession. Each sample was then analysed and print outs provided.

3.10 Determination of plasma insulin content and estimation of insulin resistance

The plasma insulin concentration was determined using the enzyme-linked immunosorbent assay (ElabScience Biotechnology, Houston, Texas USA) according to manufacturer's instructions. The immunoassay procedure used is a quantitative technique which utilizes monoclonal antibodies specific for rat insulin. Absorbencies were read off a plate reader (Multiskan Ascent, Lab System Model354, Helsinki, Finland) set at 450 nm. A standard curve was constructed and the concentrations of insulin in the samples were determined from the standard curve (see **Appendix 2** for a detailed description of the protocol). The fasting glucose concentration and insulin data were then used to compute fasting whole-body insulin sensitivity and β-cell function using the homeostasis model assessment of insulin resistance (HOMA-IR) as described by Mathews et al. (1985) using the equation:
HOMA-IR = Fasting plasma glucose (mg/dL) × fasting plasma insulin (µU/mL)] / 405.

3.11 Determination of long bone indices

The right femur and tibia were dissected out from each carcass, defleshed and soft tissues removed. The bones were then dried in an oven (Salvis®, Salvis Lab, Schweiz, Switzerland) at 50˚C for 5 days to constant mass. Each dry bone was then weighed using an electronic balance (Presica 310M electronic balance, Presica Instruments AG, Switzerland). The length of the tibia (from the tibial head to the medial malleolus) and that of the femur (from the distal femoral articular surface to the greater trochanter) were measured using a pair of digital vernier calipers (High-impact, Dejuca, South Africa). The long bone densities were calculated as described by Seedor et al. (1991) using the following formula:
bone mass to length ratio = mass of the bone (mg) / length of the bone (mm).

3.12 Determination of liver and kidney micro-morphometry

The preserved liver and kidney samples were routinely processed using the automatic tissue processor (Microm STP 120 Thermoscientific, Massachusetts, USA) and embedded in paraffin wax blocks. Two sections per rat of 3µm thick were cut using a rotary microtome after which they were mounted on glass slides. The tissue sections were then stained using haematoxylin and eosin (HE). Using a Leica ICC50 HD video camera (Leica, Wetzlar, Germany) linked to a Leica DM 500 microscope (Leica, Wetzlar, Germany), photomicrographs of the stained sections were captured and analysed using the ImageJ software. The stained sections of the liver were scored semi-quantitatively for steatosis and inflammation according to Kleiner et al. (2005) and the NASH Clinical Research Network (NASH-CRN). To determine steatosis, hepatocellular vesicular steatosis was analysed based on the total area affected and grading was done as follows: grade 0 = <5% steatosis; grade 1 = 5-33% steatosis, grade 2 = 33-66% steatosis, grade 3 = >% steatosis per camera field of the liver parenchyma. To determine inflammation, scoring was done by counting the number of inflammatory cell aggregates in the liver parenchyma (Liang et al., 2014) and graded as follows: grade 0 = none or no foci of inflammation per camera field, grade 1 = less than 2 foci per camera field, grade 2 = 2-4 foci per camera field, grade 3 = greater than 4 foci per camera field at ×20 magnification. Glomerular area and renal corpuscular measurements were quantitatively assessed from photomicrographs of stained sections using the ImageJ software at × 40 magnifications (Schneider et al., 2012).

3.13 Statistical analyses

Parametric data is presented as mean ± standard deviation (SD) while non-parametric data is presented as median and interquartile ranges (minimum, maximum). Data analysis was performed using GraphPad Prism for Windows version 6.0 (Graph Pad software, San Diego, California, USA). A repeated measures analysis of variance (ANOVA) was used for statistical analyses of weekly body masses and OGTT data while other parametric data was analysed using a one-way ANOVA. The Kruskal-Wallis test was used to analyse non-alcoholic fatty liver disease scores for steatosis and inflammation. The means of parametric data were compared using a Bonferroni *post hoc* test while the medians of non-parametric data were compared using the Dunns *post hoc* test. Statistical significance was accepted at $P<0.05$.

CHAPTER 4: RESULTS

4.1 *F. thonnigii* extract yield and phytochemical composition

4.1.1 Extract yield

The crude methanolic *Ficus thonningii* stem bark extract yield was 7.9%.

4.1.2 Phytochemical composition

The qualitative phytochemical composition of crude *Ficus thonningii* stem-bark extract is shown in Table 4.1 below.

Table 4.1: Phytochemical composition of crude methanolic *Ficus thonningii* stem-bark extracts

Phytochemical	Present/Absent
Terpenoids	present
Saponins	present
Flavonoids	present
Tannins	present

The *Ficus thonningii* stem-bark extracts contained terpenoids, saponins, flavonoids and tannins.

4.2 Glucose tolerance

The blood glucose concentration of the male rats pre- and post-gavage with an oral glucose load is shown in Figure 4.1 below.

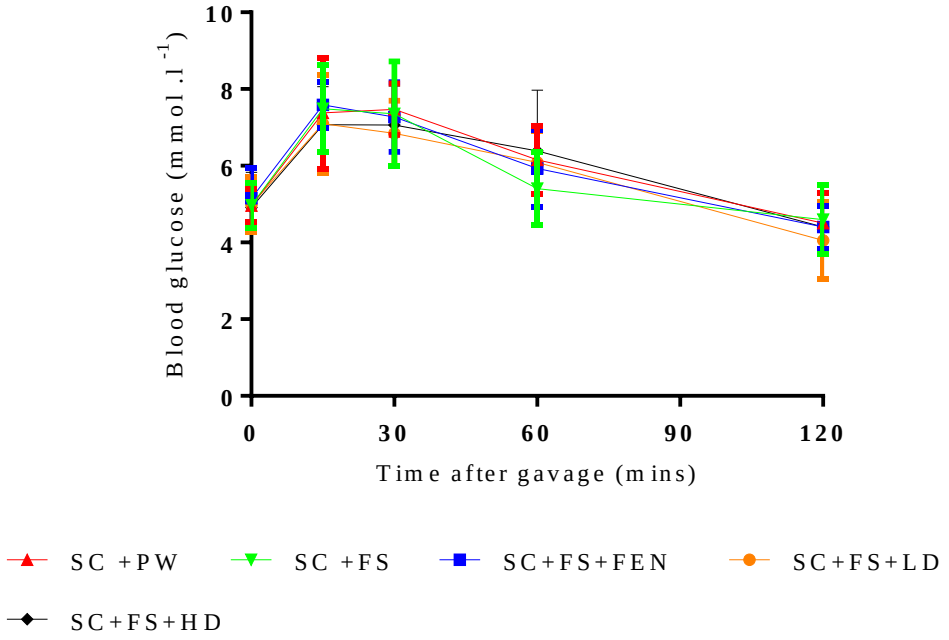


Figure 4.1: Effects of crude methanolic *F. thonningii* stem-bark extracts on glucose tolerance of male rats fed a high fructose diet

Fasting glucose concentrations of the rats were statistically similar (P>0.05) across treatments regimens. The rats' blood glucose concentration post-gavage at all-time intervals across treatment regimens were similar (P> 0.05). SC+PW = standard rat chow + plain drinking water + plain gelatine cube; SC+FS = standard rat chow + 20% fructose (FS) in drinking water (w/v) + plain gelatine cube; SC+FS+FEN = standard rat chow + 20% FS in drinking water (w/v) + fenofibrate (100 mg/kg body mass/ day); SC+FS+LD = standard rat chow + 20% FS in drinking water (w/v) + low dose *Ficus thonningii* extract (50 mg/kg body mass/ day). SC+ FS+HD = standard rat chow + 20% FS in drinking water (w/v) + high dose *Ficus thonningii* extract (500 mg/kg body mass/ day). Data presented as mean ± SD; n = 7-8.

There were no significant differences (P>0.05) in the rats' fasting glucose concentrations and blood glucose concentrations post–gavage with an oral glucose load at all intervals across the treatment regimens. Across treatment regimens, the rats' blood glucose concentration peaked 15 minutes post-gavage and returned to basal concentration at 120 minutes post-gavage.

The area under the curve for male rats calculated from oral glucose tolerance test is shown in Figure 4.2 below.

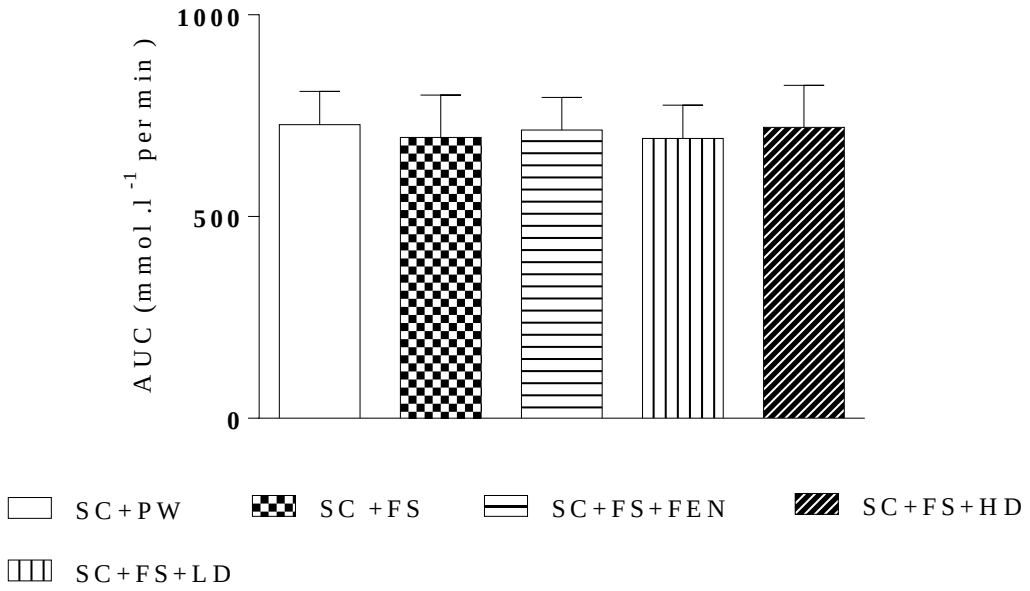


Figure 4.2: Effects of crude methanolic *Ficus thonningii* stem-bark extracts on the area under curve of oral glucose tolerance of male rats fed a high fructose diet

There was no significant difference in the total area under the curve (AUC) of oral glucose tolerance test (OGTT) for male rats across treatment regimens ($P>0.05$). SC+PW = standard rat chow + plain drinking water + plain gelatine cube; SC+FS = standard rat chow + 20 % fructose (FS) in drinking water (w/v) + plain gelatine cube; SC+FS+FEN = standard rat chow + 20% FS in drinking water (w/v) + fenofibrate (100 mg/kg body mass/ day); SC+FS+LD = standard rat chow + 20% FS in drinking water (w/v) + low dose *Ficus thonningii* extract (50 mg/kg body mass/ day). SC+ FS+HD = standard rat chow + 20% FS in drinking water (w/v) + high dose *Ficus thonningii* extract (500 mg/kg body mass/ day). Data presented as mean ± SD; n = 7-8.

The treatment regimens had no effect on tolerance to an oral glucose load.

The blood glucose concentration of the female rats pre- and post-gavage with an oral glucose load is shown in Figure 4.3 below.

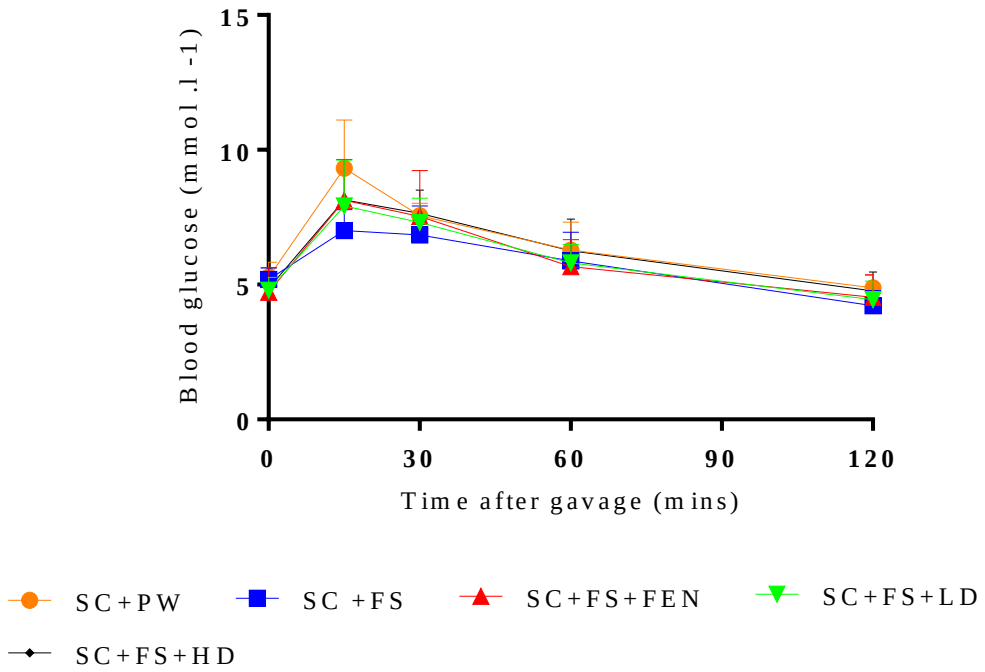


Figure 4.3: Effects of crude methanolic *F. thonningii* stem-bark extracts on glucose tolerance of female rats fed a high fructose diet

Fasting glucose concentrations were statistically similar (P> 0.05) across treatments groups. The rats' blood glucose concentrations post gavage at all-time intervals across the treatment regimens were similar (P> 0.05). SC+PW = standard rat chow + plain drinking water + plain gelatine cube; SC+FS = standard rat chow + 20 % fructose (FS) in drinking water (w/v) + plain gelatine cube; SC+FS+FEN = standard rat chow + 20% FS in drinking water (w/v) + fenofibrate (100 mg/kg body mass/ day); SC+FS+LD = standard rat chow + 20% FS in drinking water (w/v) + low dose *Ficus thonningii* extract (50 mg/kg body mass/ day). SC+ FS+HD = standard rat chow + 20% FS in drinking water (w/v) + high dose *Ficus thonningii* extract (500 mg/kg body mass/ day). Data presented as mean ± SD; n = 7-8.

There were no significant differences (P> 0.05) in the rats' fasting glucose concentrations as well as the blood glucose concentrations post-gavage at all intervals across treatment regimens.

The blood glucose concentration of the female rats across treatment peaked at 15 minutes post-gavage and returned to basal concentration at 120-min post-gavage.

The area under the curve for female rats calculated from oral glucose tolerance test is shown in Figure 4.4 below.

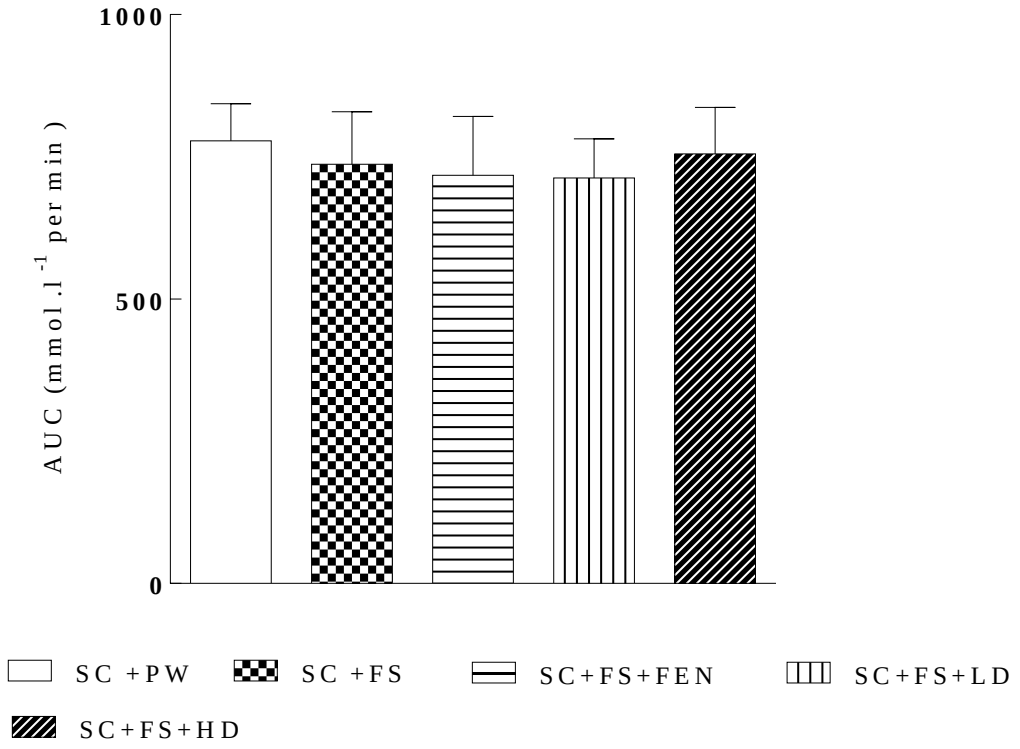


Figure 4.4: Effects of crude methanolic *Ficus thonningii* stem-bark extracts on total area under curve of oral glucose tolerance of female rats fed a high fructose diet

There was no significant difference in the total area under the curve of oral glucose tolerance test for female rats across treatment regimens ($P>0.05$). SC+PW = standard rat chow + plain drinking water + plain gelatine cube; SC+FS = standard rat chow + 20 % fructose (FS) in drinking water (w/v) + plain gelatine cube; SC+FS+FEN = standard rat chow + 20% FS in drinking water (w/v) + fenofibrate (100 mg/kg body mass/ day); SC+FS+LD = standard rat chow + 20% FS in drinking water (w/v) + low dose *Ficus thonningii* extract (50 mg/kg body mass/ day). SC+ FS+HD = standard rat chow + 20% FS in drinking water (w/v) + high dose *Ficus thonningii* extract (500 mg/kg body mass/ day. Data presented as mean ± SD; n = 7-8.

The treatment regimens had no effect on tolerance to an oral glucose load.

4.3 Effect of crude *Ficus thonningii* extracts on growth performance

4.3.1 Body mass

The induction and terminal body masses of the male rats are shown in Figure 4.5 below.

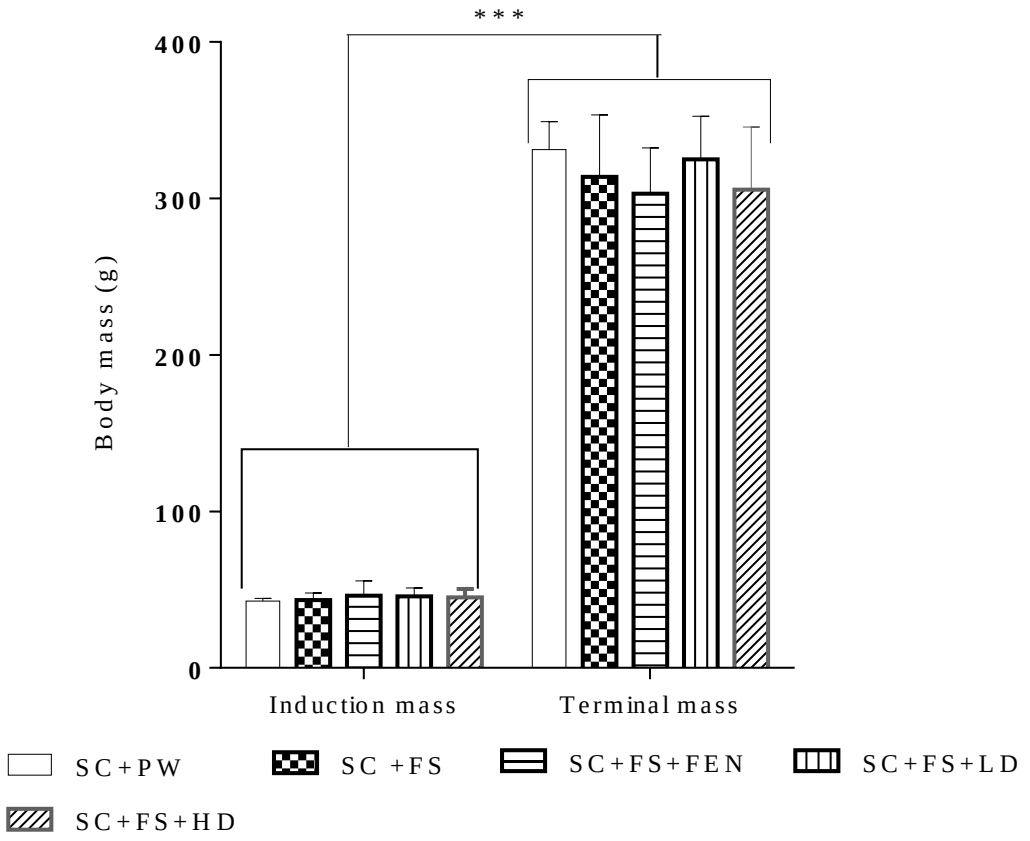


Figure 4.5: The induction and terminal body masses of male rats

*** P<0.0001. The mean induction body masses of rats were not significantly different (P>0.05). The mean terminal body masses of the rats were similar across treatment regimens (P>0.05). SC+PW = standard rat chow + plain drinking water + plain gelatine cube; SC+FS = standard rat chow + 20 % fructose (FS) in drinking water (w/v) + plain gelatine cube; SRC+FS+FEN = standard rat chow + 20% FS in drinking water (w/v) + fenofibrate (100 mg/kg body mass/ day); SC+FS+LD = standard rat chow + 20% FS in drinking water (w/v) + low dose *Ficus thonningii* extract (50 mg/kg body mass/ day. SR+ FS+HD = standard rat chow + 20% FS in drinking water (w/v) + high dose *Ficus thonningii* extract (500 mg/kg body mass/ day. Data presented at mean ± SD; n =7-8.

The induction body masses of the male rats were similar. Although treatment regimens had no effect on the terminal body masses of the male rats, the rats grew significantly (P<0.05) during the trial period.

The induction and terminal body masses of the female rats are shown in Figure 4.6 below.

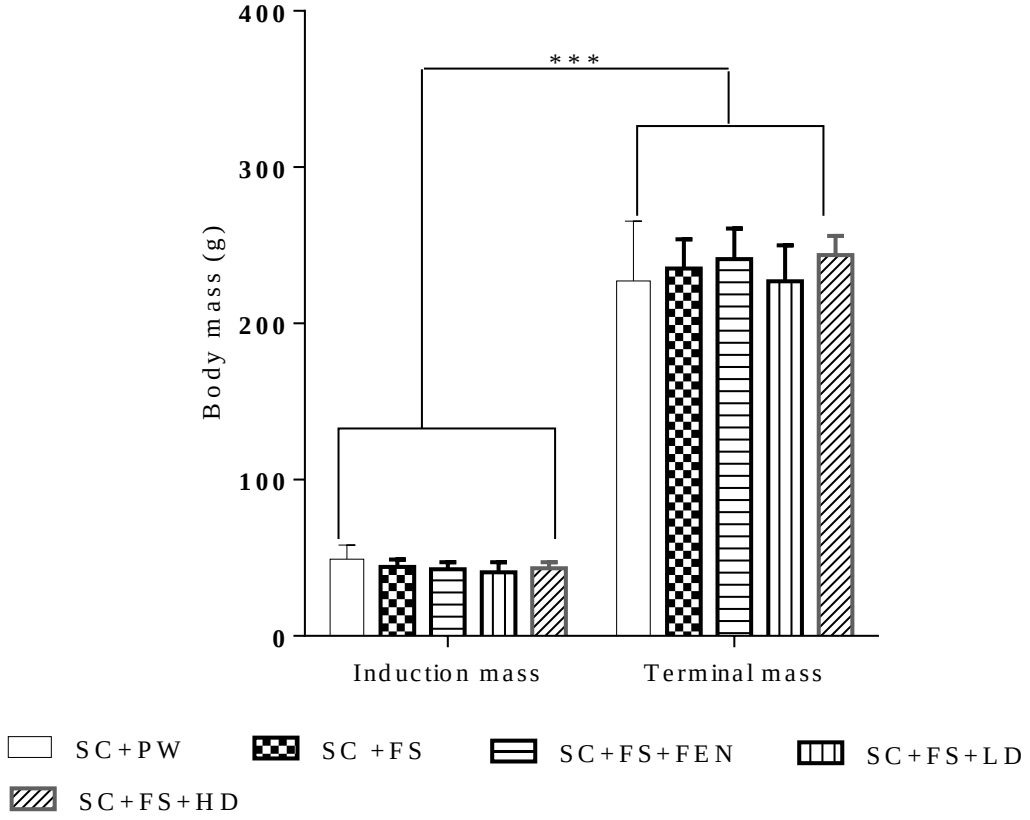


Figure 4.6: The induction and terminal body masses of the female rats

*** P<0.0001. The mean induction body masses of female rats were not significantly different (P> 0.05). The mean terminal body masses of the rats were similar across treatment regimens (P>0.05). SC+PW = standard rat chow + plain drinking water + plain gelatine cube; SC+FS = standard rat chow + 20 % fructose (FS) in drinking water (w/v) + plain gelatine cube; SC+FS+FEN = standard rat chow + 20% FS in drinking water (w/v) + fenofibrate (100 mg/kg body mass/ day); SC+FS+LD = standard rat chow + 20% FS in drinking water (w/v) + low dose *Ficus thonningii* extract (50 mg/kg body mass/ per day); SC+ FS+HD = standard rat chow + 20% FS in drinking water (w/v) + high dose *Ficus thonningii* extract (500 mg/kg body mass) per day. Data presented as mean ± SD; n = 7-8.

The induction body masses of the female rats were similar. Although treatment regimens had no effect on the terminal body masses of the female rats, the rats grew significantly (P<0.05) during the trial period.

4.3.2 Empty carcass mass

The empty carcass masses of male rats are shown in Figure 4.7 below.

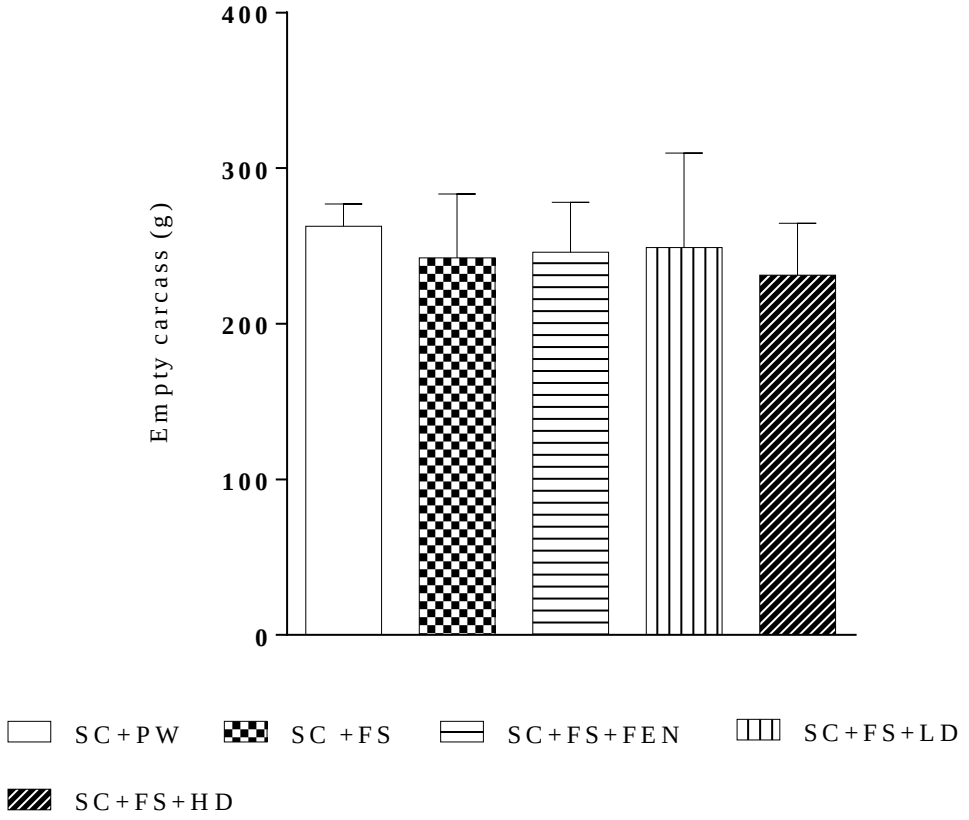


Figure 4.7: Effects of crude methanolic *F. thonningii* stem bark extracts on empty carcass masses of the male rats fed a high fructose diet

The mean empty carcass masses of male rats were similar (P>0.05) across treatment regimens. SC+PW = standard rat chow + plain drinking water + plain gelatine cube; SC+FS = standard rat chow + 20 % fructose (FS) in drinking water (w/v) + plain gelatine cube; SRC+FS+FEN = standard rat chow + 20% FS in drinking water (w/v) + fenofibrate (100 mg/kg body mass/ day); SC+FS+LD = standard rat chow + 20% FS in drinking water (w/v) + low dose *Ficus thonningii* extract (50 mg/kg body mass/day); SC+ FS+HD = standard rat chow + 20% FS in drinking water (w/v) + high dose *Ficus thonningii* extract (500mg/kg body mass/day); Data presented at mean ± SD, n = 7-8.

The empty carcass masses for male rats were similar across the treatment regimens.

The empty carcass masses of female rats are shown in Figure 4.8 below.

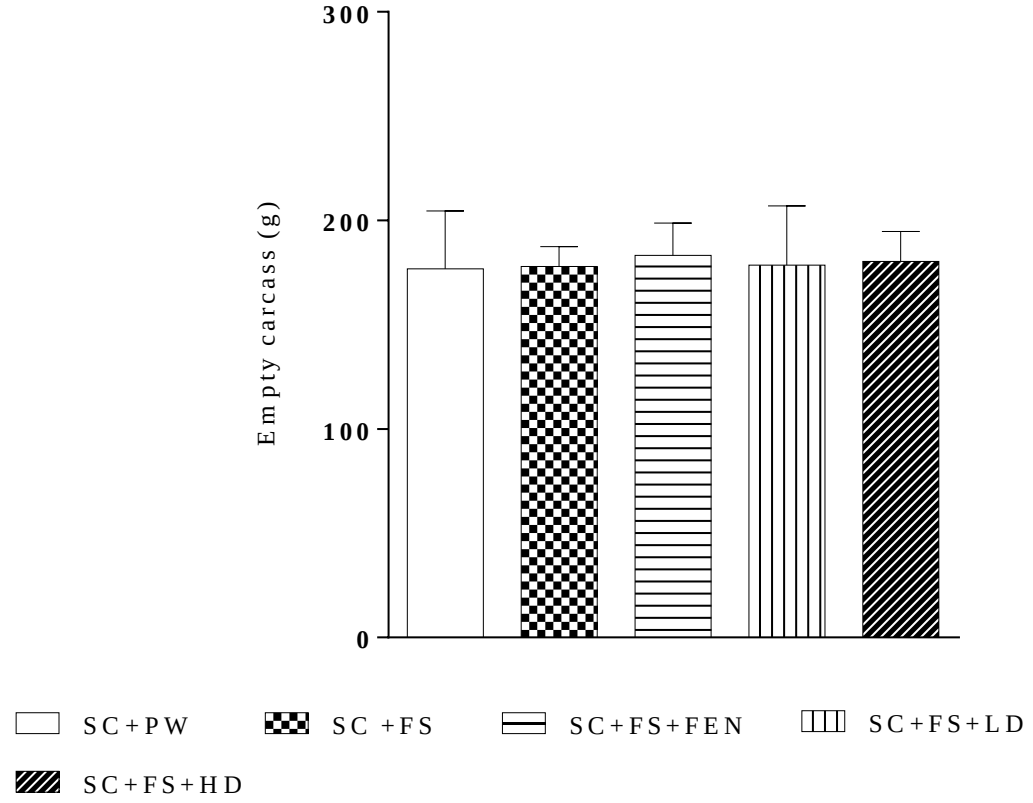


Figure 4.8: Effects of crude methanolic *F. thonningii* stem bark extracts on carcass mass of the female rats fed a high fructose diet

The mean empty carcass masses were not significantly different (P>0.05) across treatment regimens. SC+PW = standard rat chow + plain drinking water + plain gelatine cube; SC+FS = standard rat chow +20 % fructose (FS) in drinking water (w/v) + plain gelatine cube; SC+FS+FEN = standard rat chow + 20% FS in drinking water (w/v) + fenofibrate (100 mg/kg body mass/r day); SC+FS+LD = standard rat chow + 20% FS in drinking water (w/v) + low dose *Ficus thonningii* extract (50 mg/kg body mass/ day; SC+ FS+HD = standard rat chow + 20% FS in drinking water (w/v) + high dose *Ficus thonningii* extract (500 mg/kg body mass/ day. Data presented as mean ± SD; n = 7-8.

The empty carcass masses for female rats were similar across the treatments regimens.

4.3.3 Linear growth

The masses, lengths and density of tibiae and femora of male rats are shown in Table 4.2 below.

Table 4.2: Effects of crude methanolic *Ficus thonningii* stem-bark extract on bone indices of male rats fed a high-fructose diet

Parameter	SC+PW	SC+FS	SC+FS+FEN	SC+FS+LD	SC+FS+HD	Significance level
Tibia						
Mass (mg)	510.90±71.72[a]	435.40±35.77[a]	479.30±46.87[a]	492.80±41.47[a]	489.40±51.37[a]	ns
Length (mm)	40.39±2.02[a]	39.73±0.99[a]	39.06±1.69[a]	39.55±1.76[a]	39.45±1.50[a]	ns
Density (mg/mm)	12.59±1.22[a]	11.03±0.82[a]	12.15±1.00[a]	12.51±0.77[a]	13.12±2.40[a]	ns
Femur						
Mass (mg)	647.00±70.73[b]	539.40±48.14[ab]	524.80±77.06[a]	589.00±69.37[ab]	521.00±57.33[a]	*
Length (mm)	34.21±1.49[a]	34.29±2.23[a]	31.99±1.88[a]	34.57±2.91[a]	32.76±2.14[a]	ns
Density (mg/mm)	18.88±3.07[b]	16.21±0.78[a]	16.41±1.46[a]	17.45±0.92[ab]	16.01±0.74[a]	*

ns = not significant, P>0.05; *P<0.05; [ab]Within row means with different superscripts are significantly different at P<0.05. Male rats administered with SC+PW had significantly heavier femora (P<0.05) compared to femora from rats fed a high fructose diet with fenofibrate as an intervention and those fed a high fructose diet with the high dose *F. thonningii* extract as an intervention. The density of the femora from rats fed the standard rat chow and plain drinking water were significantly higher (P<0.05) compared to that of femora from rats fed a high fructose diet, high fructose diet with fenofibrate and or high dose *F. thonningii* extract as an intervention, respectively. Tibiae masses, lengths and density and femora lengths of male rats were similar across treatment regimens (P>0.05). SC+PW = standard rat chow + plain drinking water + plain gelatine cube; SC+FS = standard rat chow + 20% fructose (FS) in drinking water (w/v) + plain gelatine cube; SC+FS+FEN = standard rat chow + 20% FS in drinking water (w/v) + fenofibrate (100 mg/kg body mass/ day); SC+FS+LD = standard rat chow + 20% FS in drinking water (w/v) + low dose *Ficus thonningii* extract (50 mg/kg body mass/ day). SC+ FS+HD = standard rat chow + 20% FS in drinking water (w/v) + high dose *Ficus thonningii* extract (500 mg/kg body mass/ day. Data presented as mean ± SD; n = 7-8.

The male rats that were fed a high-fructose diet with fenofibrate or high dose of *F. thonningii* extract as interventions had significantly lighter femora compared to femora from control rats ($P<0.05$). However, there were no significant differences in the femora masses of rats that were fed a control diet and a high-fructose diet with a low dose of *F. thonningii* extract as an intervention compared to rats in the other treatment groups ($P<0.05$). While the rats fed a control, diet had denser femora compared to those fed a high-fructose diet alone or a high-fructose diet with fenofibrate or a high dose of *F. thonningii* as interventions ($P<0.05$), the tibiae masses, lengths and densities and femora lengths were similar across treatment groups ($P>0.05$).

The tibiae and femora masses, lengths and density of female rats are shown in table 4.3 below.

Table 4.3: Effect of crude methanolic *Ficus thonningii* stem-bark extract on bone indices of female rats fed a high-fructose diet

Parameter						Significance level
	SC+PW	SC+FS	SC+FS+FEN	SC+FS+LD	SC+FS+HD	
Tibia						
Mass (mg)	449.80±32.20[a]	416.40±73.66[a]	377.80±43.64[a]	396.50±32.97[a]	380.30±55.81[a]	ns
Length (mm)	39.11±1.79[a]	37.52±2.15[ab]	37.30±2.44[ab]	37.21±1.57[ab]	35.64±2.97[b]	*
Density (mg/mm)	11.42±0.87[a]	11.02±1.33[a]	10.41±1.02[a]	10.61±1.07[a]	10.82±1.02[a]	ns
Femur						
Mass (mg)	519.80±51.38[a]	515.00±86.02[a]	515.0±86.02[a]	468.20±60.59[a]	512.80±11.12[a]	ns
Length (mm)	32.48±0.87[a]	31.44±1.01[a]	31.15±2.68[a]	31.30±2.34[a]	31.86±1.29[a]	ns
Density (mg/mm)	17.36±0.98[a]	16.59±1.42[ab]	17.02±1.55[ab]	15.07±1.23[b]	16.05±0.56[ab]	*

ns = not significant, P > 0.05; *P ≤ 0.05. [ab]Within row means with different superscripts are significantly different at P<0.05. Rats fed a high fructose diet with a high dose of *F.thonningii* extract as an intervention had significantly shorter tibiae compared to the rats fed the control diet (P< 0.05). Rats fed a high fructose diet with the low dose of *F. thonningii* as an intervention had less dense femora compared to those fed the control diet (P<0.05). Tibiae density indices and femora masses and lengths were similar across treatment groups (P>0.05). SC+PW = standard rat chow + plain drinking water + plain gelatine cube; SC+FS = standard rat chow + 20 % fructose (FS) in drinking water (w/v) + plain gelatine cube; SC+FS+FEN = standard rat chow + 20% FS in drinking water (w/v) + fenofibrate (100 mg/kg body mass/ day); SC+FS+LD = standard rat chow + 20% FS in drinking water (w/v) + low dose *Ficus thonningii* extract (50 mg/kg body mass/ day). SC+ FS+HD = standard rat chow + 20% FS in drinking water (w/v) + high dose *Ficus thonningii* extract (500 mg/kg body mass/ day. Data presented as mean ± SD; n = 7-8.

Females rats fed a high fructose diet with a high dose of *F. thonningii* as an intervention had significantly shorter tibiae ($P<0.05$) compared to that of rats fed the standard rat chow and plain drinking water. The femora of female rats fed a high fructose diet with the low dose crude methanolic *F. thonningii* stem bark extract as an intervention were less dense compared to that of counterparts administered the control treatment regimen.

4.4 Gastrointestinal tract viscera macro-morphometry

The effects of crude methanolic *F.thonningii* stem-bark extracts on the absolute and relative masses as well as the lengths of the GIT viscera of male rats fed a high-fructose diet are shown in Table 4.4.

The masses of the rats' small and large intestines as well as the lengths of both the small and large intestines of the rats were also similar were similar ($P>0.05$) across treatment regimens. The absolute large intestinal mass and length of rats administered the high dose crude methanolic *F. thonningii* stem bark extracts were lighter and shorter ($P<0.05$) compared to that of counterparts administered control.

Table 4.4: Effect of crude methanolic *F. thonningii* stem-bark extract on GIT viscera macro-morphometry of male rats fed a high-fructose diet

Viscera	SC+PW	SC+FS	SC+FS+FEN	SC+FS+LD	SC+FS+HD	Significance level
SI length (mm)	1388.00±69.02[a]	1313.00±93.04[a]	1308.00±70.66[a]	1305.00±69.22[a]	1303.00±50.21[a]	ns
Mass (g)	8.63±0.80[a]	8.87±1.01[a]	8.49±1.73[a]	9.82±1.57[a]	8.96±0.59[a]	ns
%BM	2.60±0.22[a]	2.85±0.41[a]	2.86±0.76[a]	2.66±0.23[a]	2.97±0.37[a]	ns
TLr (g/mm)	21.40±2.19[a]	22.30±2.72[a]	21.80±4.45[a]	22.30±3.80[a]	22.70±1.36[a]	ns
LI mass (mm)	226.00±13.00[a]	216.00±9.70[ab]	216.00±16.20[ab]	206.00±19.30[ab]	202.00±12.30[b]	*
length (g)	1.84±0.16[a]	1.58±0.29[ab]	1.58±0.29[ab]	1.61±0.10[ab]	1.47±0.17[b]	**
%BM	0.56±0.05[a]	0.50±0.04[a]	0.53±0.11[a]	0.48±0.04[a]	0.49±0.07[a]	ns
TLr (g/mm)	3.26±0.72[a]	3.55±0.60[a]	3.19±0.14[a]	3.29±0.65[a]	3.57±0.64[a]	ns
Stomach mass (g)	1.65±0.22[a]	1.50±0.12[a]	1.58±0.17[a]	1.58±0.18[a]	1.53±0.13[a]	ns
%BM	0.49±0.06[a]	0.48±0.05[a]	0.52±0.07[a]	0.47±0.05[a]	0.50±0.06[a]	ns
TLr (g/mm)	4.09±0.68[a]	3.77±0.29[a]	4.04±0.40[a]	4.01±0.50[a]	3.89±0.32[a]	ns
Caecum mass (g)	1.40±0.22[a]	1.20±0.21[a]	1.13±0.10[a]	1.18±0.15[a]	1.22±0.10[a]	ns
%BM	0.42±0.07[a]	0.39±0.08[a]	0.38±0.08[a]	0.35±0.03[a]	0.40±0.07[a]	ns
TLr (g/mm)	3.46±0.57 [a]	3.02±0.56[a]	2.89±0.49[a]	2.99±0.34[a]	3.11±0.57[a]	ns

ns = not significant, P > 0.05; * P < 0.05; ** P<0.01. [ab]Within row means with different superscripts are significantly different at P<0.05. Rats fed the high fructose diet with the high dose *F. thonningii* extract as an intervention had significantly shorter (P<0.01) and lighter (P<0.05) large intestines compared to those from counterparts fed the control diet. SC+PW = standard rat chow + plain drinking water + plain gelatine cube; SC+FS = standard rat chow + 20 % fructose (FS) in drinking water (w/v) + plain gelatine cube; SC+FS+FEN = standard rat chow + 20% FS in drinking water (w/v) + fenofibrate (100 mg/kg body mass/ day); SC+FS+LD = standard rat chow + 20% FS in drinking water (w/v) + low dose *Ficus thonningii* extract (50 mg/kg body mass/ day). SC+ FS+HD = standard rat

chow + 20% FS in drinking water (w/v) + high dose *Ficus thonningii* extract (500 mg/kg body mass/ day); LI = large intestines; SI= small intestines %BM = percent of body; TLr = relative to tibia length; Data presented as mean ± SD; n = 7-8.

The effects of crude methanolic *F. thonningii* stem-bark extracts on the absolute and relative masses as well as the lengths of the GIT viscera of female rats fed a high-fructose diet are shown in Table 4.5 below.

Table 4.5: Effect of crude methanolic *Ficus thonningii* stem-bark extract on GIT visceral macro-morphometry of female rats fed a high-fructose diet

Viscera	SC+PW	SC+FS	SC+FS+FEN	SC+FS+LD	SC+FS+HD	Significance level
SI mass (g)	6.02±1.17[b]	6.69±0.63[ab]	7.47±0.58[a]	6.96±0.60[ab]	7.35±0.80[a]	*
%BM	2.51±0.41[b]	2.85±0.16[ab]	3.12±0.35[a]	3.08±0.26[a]	3.02±0.31[a]	*
TLr (g/mm)	14.66±3.85[b]	17.83±1.09[ab]	20.04±0.88[a]	18.73±1.89[a]	20.75±2.87[a]	***
LI length (mm)	194.30±10.18[a]	198.60±15.74[a]	189.30±7.32[a]	180.70±16.44[a]	190.7±13.97[a]	ns
mass (g)	1.27±0.25[a]	1.33±0.21[a]	1.19±0.10[a]	1.22±0.21[a]	1.26±0.10[a]	ns
%BM	0.56± 0.09[a]	0.56±0.07[a]	0.49±0.05[a]	0.54±0.06[a]	0.52±0.09[a]	ns
TLr (g/mm)	3.26± 0.72[a]	3.55±0.60[a]	3.19±0.14[a]	3.29±0.65[a]	3.57±0.64[a]	ns
Stomach mass (g)	1.29±0.07[a]	1.29±0.14[a]	1.36±0.17[a]	1.28±0.15[a]	1.34±0.12[a]	ns
%BM	0.58±0.07[a]	0.55±0.04[a]	0.57±0.07[a]	0.57±0.07[a]	0.55±0.05[a]	ns
TLr (g/mm)	3.32±0.28[a]	3.43±0.29[a]	3.65±0.43[a]	3.44±0.46[a]	3.77±0.37[a]	ns
Caecum mass (g)	1.40±0.20[a]	1.20±0.22[a]	1.13±0.20[a]	1.18±0.15[a]	1.22±0.20[a]	ns
%BM	0.48±0.08[a]	0.44±0.07[a]	0.41±0.07[a]	0.42±0.08[a]	0.39±0.05[a]	ns
TLr (g/mm)	2.83±0.73[a]	2.76±0.50[a]	2.66±0.50[a]	2.55±0.52[a]	2.69±0.43[a]	ns

ns = not significant, P>0.05; *P<0.05; ***P < 0.0001. [ab]Within row means with different superscripts are significantly different at P<0.05. Rats fed the control diet had significantly lighter (P<0.05) small intestines (percent body mass and relative to tibia length) compared to that of rats fed a high-fructose diet with fenofibrate and or a high dose of *F. thonningii* extract as an intervention, respectively. Treatment regimens had no effect of the large intestine, stomach and caeca masses of the rats. SC+PW = standard rat chow + plain drinking water + plain gelatine cube; SC+FS = standard rat chow + 20 % fructose (FS) in drinking water (w/v) + plain gelatine cube; SC+FS+FEN = standard rat chow + 20% FS in drinking water (w/v) + fenofibrate (100 mg/kg body mass/ day); SC+FS+LD = standard rat chow + 20% FS in drinking water (w/v) + low dose *Ficus thonningii* extract (50 mg/kg body mass/ day). SC+ FS+HD = standard rat chow + 20% FS in drinking water (w/v) + high dose *Ficus thonningii* extract (500 mg/kg body mass/ day); LI = large intestines; SI= small intestines; %BM = percent of body mass; TLr = relative to tibia length. Data presented as mean ± SD; n = 7-8.

Female rats fed a high fructose diet with fenofibrate or a high dose of *F. thonningii* as an intervention had significantly heavier ($P< 0.05$) small intestines compared to the intestines from their female counterparts fed the control diet. Relative to tibia length and body mass, rats fed a high fructose diet with fenofibrate, a high and low dose of *F. thonningii* as interventions had significantly heavier small intestines ($P< 0.05$) compared to those fed a control diet. Small intestine lengths and absolute and relative (body mass and tibia length) and masses of the large intestines, stomachs and caeca were similar across treatment groups ($P > 0.05$).

4.5 Macro-morphometry of other viscera

The effects of crude methanolic *F. thonningii* stem-bark extracts on the absolute and relative masses of the other viscera of male rats fed a high-fructose diet are shown in Table 4.6 below.

Treatment regimens had no effect on the masses of the hearts, pancreata, visceral fat pad and epididymal fat of the rats.

Table 4.6: Effect of crude methanolic *Ficus thonningii* stem-bark extract on the macro-morphometry of other viscera from male rats fed a high-fructose diet

Organs	SC+PW	SC+FS	SC+FS+FEN	SC+FS+LD	SC+FS+HD	Significance level
Heart (g)	1.3±0.09 [a]	1.46±0.50[a]	1.32±0.16[a]	1.36±0.09[a]	1.22±0.18[a]	ns
%BM	0.39±0.03[a]	0.47±0.17[a]	0.44±0.03[a]	0.41±0.03[a]	0.34±0.03[a]	ns
TLr (g/mm)	3.23±0.343[a]	3.66±1.25[a]	3.38±0.27[a]	3.45±0.32[a]	3.09±0.44[a]	ns
Pancreas (g)	1.26±0.17[a]	1.20±0.18[a]	1.15±0,24[a]	1.17±0.19[a]	1.13±0.22[a]	ns
%BM	0.38±0.05[a]	0.39±0.05[a]	0.38±0.09[a]	0.46±0.04[a]	0.37±0.07[a]	ns
TLr (g/mm)	0.38±0.05[a]	0.39±0.05[a]	0.38±0.09[a]	0.46±0.04[a]	0.37±0.07[a]	ns
VFP (g)	6.95±1.56[a]	6.48±2.06[a]	6.66±2.57[a]	8.82±2.76[a]	6.57±2.91[a]	ns
%BM	2.10±0.48[a]	2.04±0.45[a]	2.18±0.79[a]	2.57±0.55[a]	2.09±0.67[a]	ns
EFP (g)	2.32±0.44[a]	2.03±0.40[a]	2.12±0.60[a]	2.74±0.61[a]	2.02±0.90[a]	ns
%BM	0.70±0.12[a]	0.65±0.09[a]	0.65±0.18[a]	0.81±0.11[a]	0.64±0.22[a]	ns

ns = not significant, P> 0.05; *P ≤ 0.05. ** P < 0.01; [ab]Within row means with different superscripts are significantly different at P<0.05. There were no significant differences (P>0.05) in the masses of the hearts, pancreata, visceral fat pad and epididymal fat pad masses of the rats across treatment regimens. SC+PW = standard rat chow + plain drinking water + plain gelatine cube; SC+FS = standard rat chow + 20 % fructose (FS) in drinking water (w/v) + plain gelatine cube; SC+FS+FEN = standard rat chow + 20% FS in drinking water (w/v) + fenofibrate (100 mg/kg body mass/ day); SC+FS+LD = standard rat chow + 20% FS in drinking water (w/v) + low dose *Ficus thonningii* extract (50 mg/kg body mass/ day). SC+ FS+HD = standard rat chow + 20% FS in drinking water (w/v) + high dose *Ficus thonningii* extract (500 mg/kg body mass/ day); VFP = Visceral fat pad; EFP = Epididymal fat pad; %BM = percent of body mass; TLr = relative to tibia length. Data presented as mean ± SD; n = 7-8.

The effects of crude methanolic *F. thonningii* stem-bark extracts on the absolute and relative masses of the other viscera of female rats fed a high-fructose diet are shown in Table 4.7 below.

There were no significant differences in the masses of the hearts, pancreata and visceral fat of the female rats across treatment regimens.

Table 4.7: Effect of crude methanolic *Ficus thonningii* stem-bark extract on the macro-morphometry of other viscera from female rats fed a high-fructose diet

Parameter	SRC+PW	SRC+FS	SRC+FS+FEN	SRC+FS+LD	SRC+FS+HD	Significance level
Heart (g)	0.97±0.13 [a]	1.00± 0.05[a]	1.06±0.06[a]	0.95±0.08[a]	1.02±0.05[a]	ns
%BM	0.43±0.03[a]	0.42±0.02[a]	0.44±0.04[a]	0.42± 0.03[a]	0.42± 0.03[a]	ns
TLr (g/mm)	2.48±0.39[a]	2.66±0.19[a]	2.84±0.21[a]	2.57±0.28[a]	2.88±0.28[a]	ns
Pancreas (g)	0.91±0.26[a]	0.98±0.27[a]	1.04±0.23[a]	1.09± 0.23[a]	1.23± 0.14[a]	ns
%BM	0.40± 0.01[a]	0.41±0.10[a]	0.43± 0.08[a]	0.48± 0.09[a]	0.50± 0.06[a]	ns
TLr (g/mm)	2.35±0.72[a]	2.60±0.70[a]	2.80±0.58[a]	2.95±0.70[a]	3.01±0.491[a]	ns
VFP (g)	6.12±5.17[a]	9.56±1.96[a]	7.52±3.74[a]	7.96±2.38[a]	8.93±1.81[a]	ns
%BM	2.47±1.76[a]	4.05±0.74[a]	3.14±1.57[a]	3.46±0.81[a]	3.65±0.65[a]	ns

ns = not significant, P> 0.05; * P ≤ 0.05; [ab]Within row means with different superscripts are significantly different at P<0.05. The masses of the hearts, pancreata, and visceral fat of the rats were similar (P>0.05) across treatment regimens. SC+PW = standard rat chow + plain drinking water + plain gelatine cube; SC+FS = standard rat chow + 20 % fructose (FS) in drinking water (w/v) + plain gelatine cube; SC+FS+FEN = standard rat chow + 20% FS in drinking water (w/v) + fenofibrate (100 mg/kg body mass/ day); SC+FS+LD = standard rat chow + 20% FS in drinking water (w/v) + low dose *F. thonningii* extract (50 mg/kg body mass/ day). SC+ FS+HD = standard rat chow + 20% FS in drinking water (w/v) + high dose *F. thonningii* extract (500mg/kg body mass/day); VFP = Visceral fat pad. %BM = percent of body mass; TLr = relative to tibia length. Data presented as mean ± SD; n = 7-8.

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4.6 Packed cell volume and erythrocyte osmotic fragility

The effects of crude methanolic *F. thonningii* stem-bark extracts on the packed cell volume of the male rats is shown in figure 4.9 below

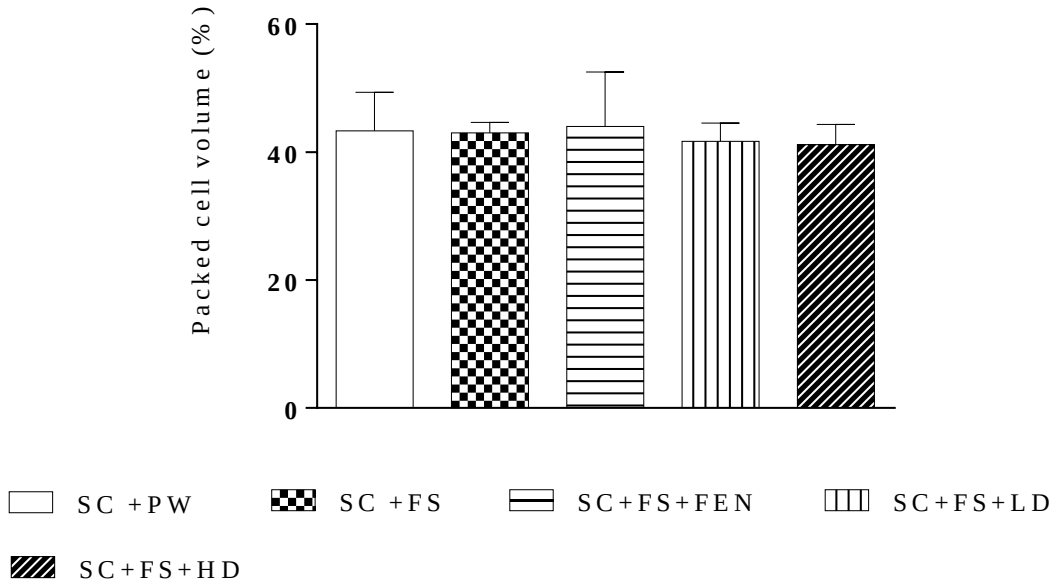


Figure 4.9: Effects of crude methanolic *Ficus thonningii* stem-bark extracts on packed cell volume of male rats fed a high fructose diet

The rats' blood mean packed cell volumes were statistically similar (P>0.05) across treatment regimens. SC+PW = standard rat chow + plain drinking water + plain gelatine cube; SC+FS = standard rat chow + 20% fructose (FS) in drinking water (w/v) + plain gelatine cube; SC+FS+FEN = standard rat chow + 20% FS in drinking water (w/v) + fenofibrate (100 mg/kg body mass/day); SC+FS+LD = standard rat chow + 20% FS in drinking water (w/v) + low dose *Ficus thonningii* extract (50 mg/kg body mass/ day). SC+ FS+HD = standard rat chow + 20% FS in drinking water (w/v) + high dose *Ficus thonningii* extract (500mg/kg body mass/ day). Data presented as mean ± SD; n = 7-8.

There were no significant differences (P>0.05) in the haematocrit of male rats across treatment regimens.

The effects of crude methanolic *F. thonningii* stem-bark extracts on the packed cell volume of the female rats is shown in figure 4.10 below

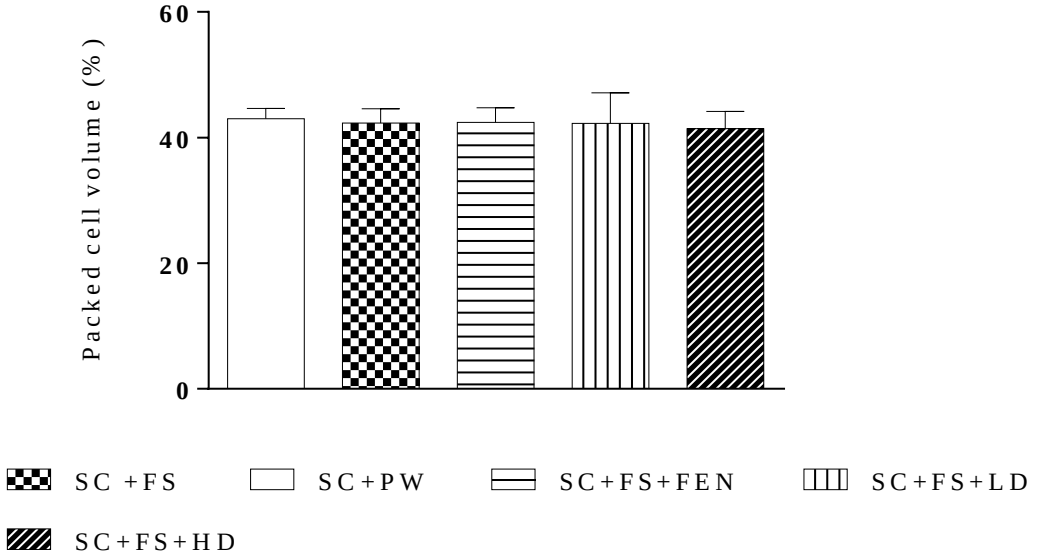


Figure 4.10: Effects of crude methanolic *Ficus thonningii* stem-bark extracts on packed cell volume female rats fed a high fructose diet

The rats' blood mean packed cell volumes were statistically similar (P>0.05) across treatment regimens. SC+PW = standard rat chow + plain drinking water + plain gelatine cube; SC+FS = standard rat chow + 20% fructose (FS) in drinking water (w/v) + plain gelatine cube; SC+FS+FEN = standard rat chow + 20% FS in drinking water (w/v) + fenofibrate (100 mg/kg body mass/ day); SC+FS+LD = standard rat chow + 20% FS in drinking water (w/v) + low dose *Ficus thonningii* extract (50 mg/kg body mass/ day). SC+ FS+HD = standard rat chow + 20% FS in drinking water (w/v) + high dose *Ficus thonningii* extract (500 mg/kg body mass/ day). Data presented as mean ± SD; n = 7-8.

There were no significant differences (P>0.05) in the haematocrit of female rats across the treatment regimens.

The effects of crude methanolic *F. thonningii* stem-bark extracts on the fragilograms of the male rats are shown in Figure 4.11 below.

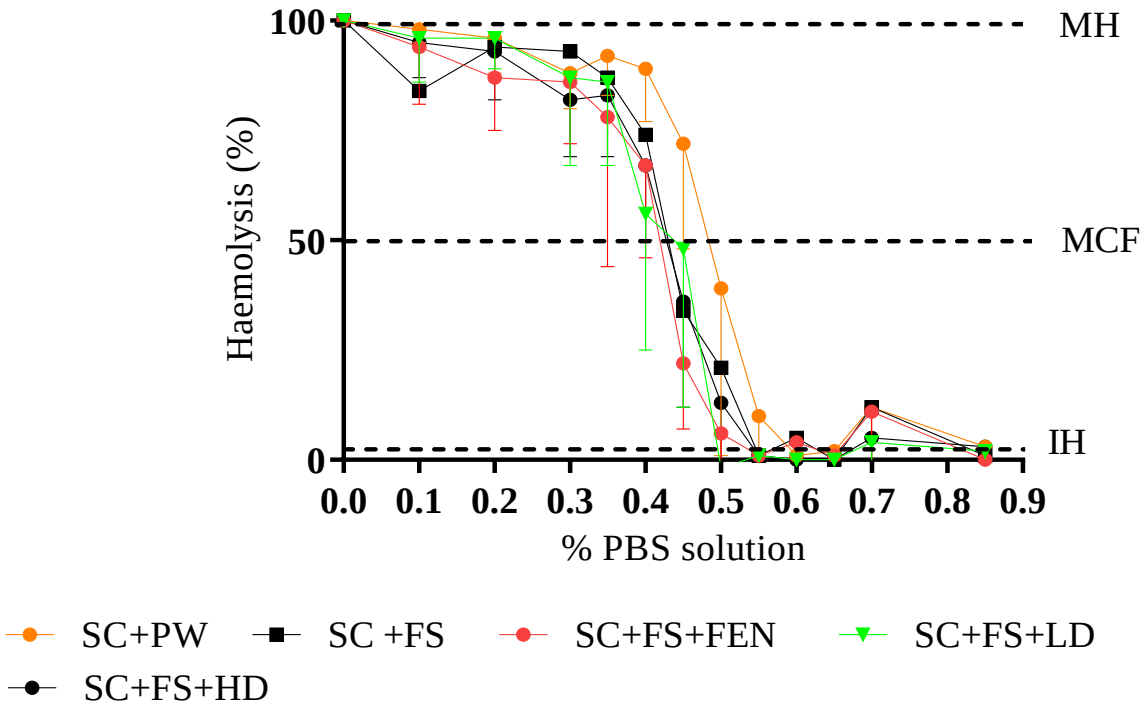


Figure 4.11: Effects of crude methanolic *Ficus thonningii* stem-bark extracts on red blood cell osmotic fragility of male rats fed a high fructose diet

The haemolysis of the rats' erythrocytes was similar across treatment regimens (P>0.05). SC+PW = standard rat chow + plain drinking water + plain gelatine cube; SC+FS = standard rat chow + 20% fructose (FS) in drinking water (w/v) + plain gelatine cube; SC+FS+FEN = standard rat chow + 20% FS in drinking water (w/v) + fenofibrate (100 mg/kg body mass/ day); SC+FS+LD = standard rat chow + 20% FS in drinking water (w/v) + low dose *Ficus thonningii* extract (50 mg/kg body mass/ day). SC+ FS+HD = standard rat chow + 20% FS in drinking water (w/v) + high dose *Ficus thonningii* extract (500 mg/kg body mass/ day). Data presented as mean ± SD; n = 7-8.

There was no significant difference in haemolysis of the male rats' erythrocytes across treatment regimens. The minimum (4%) haemolysis occurred at PBS concentration 0.55. to 0.85. The maximal (100%) haemolysis occurs at PBS concentration 0.00 to 0.10.

The effects of crude methanolic *F. thonningii* stem-bark extracts on the fragilograms of the female rats are shown in Figure 4.12 below.

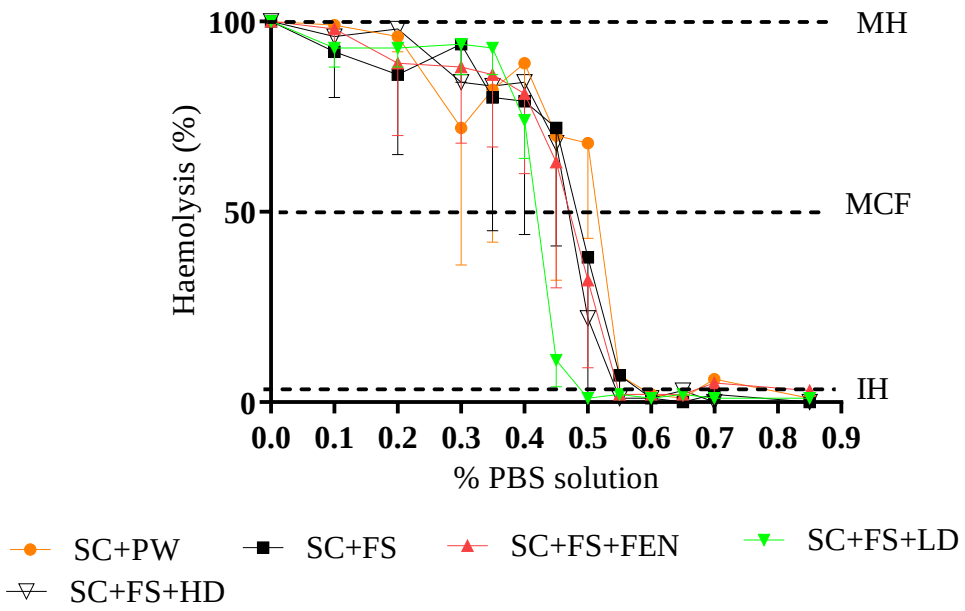


Figure 4.12: Effects of crude methanolic *Ficus thonningii* stem-bark extracts on red blood cell osmotic fragility of female rats fed a high fructose diet

The haemolysis of the rats' erythrocytes was similar across treatment regimens (P>0.05). SC+PW = standard rat chow + plain drinking water + plain gelatine cube; SC+FS = standard rat chow + 20% fructose (FS) in drinking water (w/v) + plain gelatine cube; SC+FS+FEN = standard rat chow + 20% FS in drinking water (w/v) + fenofibrate (100 mg/kg body mass/ day); SC+FS+LD = standard rat chow + 20% FS in drinking water (w/v) + low dose *F. thonningii* extract (50 mg/kg body mass/day). SC+ FS+HD = standard rat chow + 20% FS in drinking water (w/v) + high dose *F. thonningii* extract (500mg/kg body mass/ day). Data presented as means ± SD; n = 7-8.

There was no significant difference in haemolysis of the female rats' erythrocytes across treatment regimens. The minimum (4%) haemolysis occurred at PBS concentration 0.50. to 0.60. The maximal (100%) haemolysis occurs at PBS concentration 0.00 to 0.10.

4.7 Hepatic lipid content

The effects of crude methanolic *F.thonningii* stem-bark extracts on the hepatic lipid content of male rats fed a high-fructose diet are shown in Figure 4.13 below.

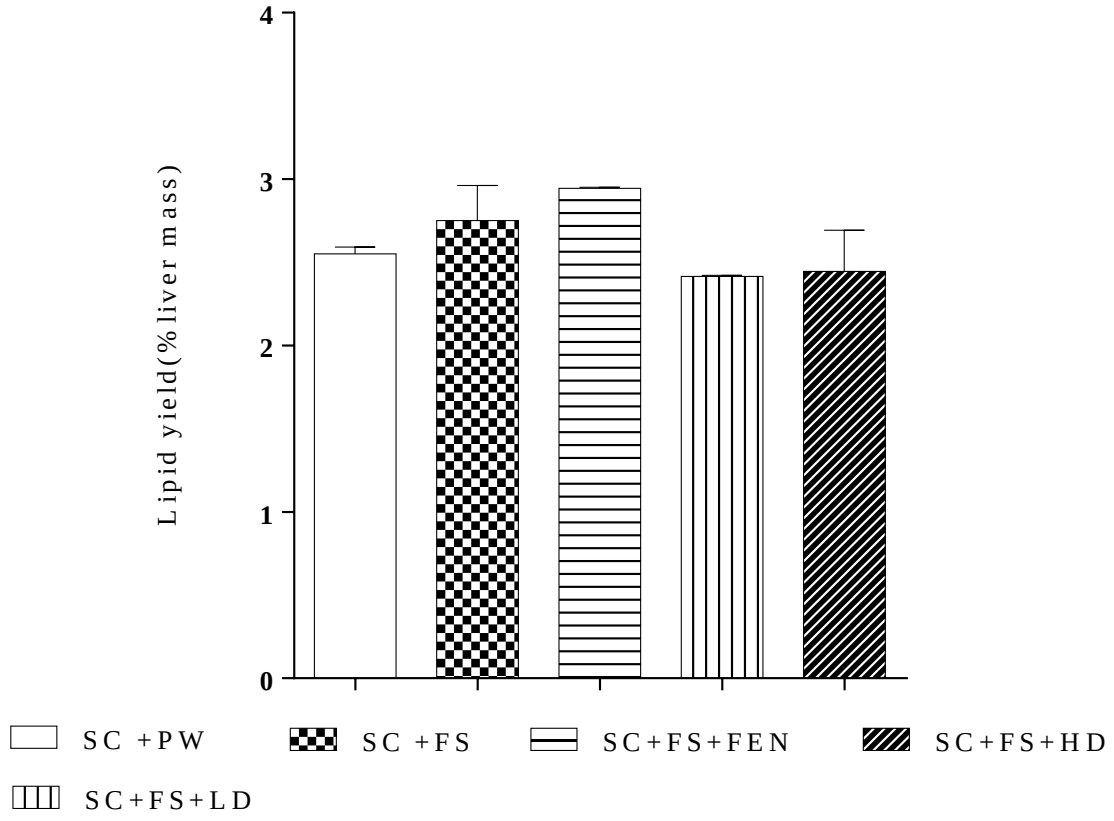


Figure 4.13: Effect of crude methanolic *F. thonningii* stem bark extracts on liver lipid content of male rats fed a high fructose diet

The rats' liver lipid content was statistically similar (P>0.05) across treatment regimens. SC+PW = standard rat chow + plain drinking water + plain gelatine cube; SC+FS = standard rat chow + 20% fructose (FS) in drinking water (w/v) + plain gelatine cube; SC+FS+FEN = standard rat chow + 20% FS in drinking water (w/v) + fenofibrate (100 mg/kg body mass/ day); SC+FS+LD = standard rat chow + 20% FS in drinking water (w/v) + low dose *Ficus thonningii* extract (50 mg/kg body mass/ day). SC+ FS+HD = standard rat chow + 20% FS in drinking water (w/v) + high dose *Ficus thonningii* extract (500 mg/kg body mass/ day). Data presented as mean ± SD; n = 7-8.

There were no significant differences (P>0.05) in the hepatic lipid content of the rats across treatment regimens.

The effects of crude methanolic *F. thonningii* stem-bark extracts on the hepatic lipid content of female rats fed a high-fructose diet are shown in Figure 4.14 below.

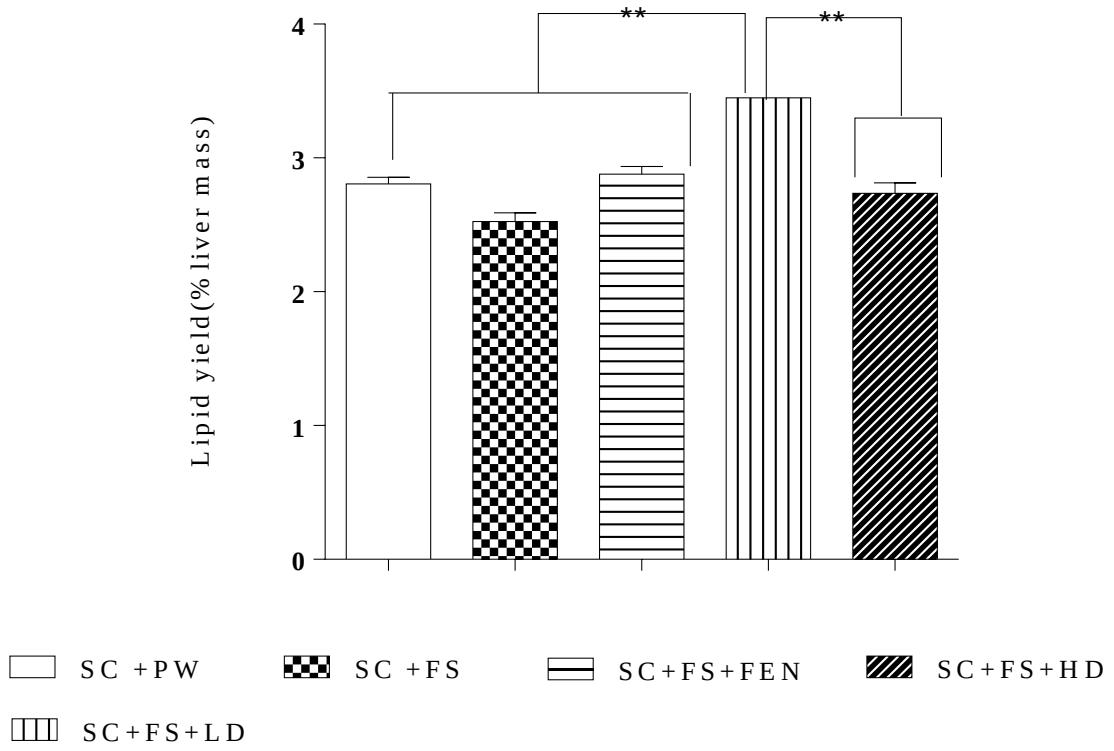


Figure 4.14: Effect of crude methanolic *F. thonningii* stem bark extracts on liver lipid content of female rats fed a high-fructose diet

**P<0.001. Rat fed a high fructose diet with a low dose of *F. thonningii* as an intervention had significantly higher (P < 0.0001) liver lipid content compared to rats under all other treatment regimens. SC+PW = standard rat chow + plain drinking water + plain gelatine cube; SC+FS = standard rat chow + 20% fructose (FS) in drinking water (w/v) + plain gelatine cube; SC+FS+FEN = standard rat chow + 20% FS in drinking water (w/v) + fenofibrate (100 mg/kg body mass/ day); SC+FS+LD = standard rat chow + 20% FS in drinking water (w/v) + low dose *Ficus thonningii* extract (50 mg/kg body mass/ day). SC+ FS+HD = standard rat chow + 20% FS in drinking water (w/v) + high dose *F. thonningii* extract (500 mg/kg body mass/ day). Data presented as mean ± SD; n = 7-8.

The rats fed a high fructose diet with a low dose of the crude methanolic *F. thonningii* stem-bark as an intervention had significantly higher (P < 0.001) liver lipid content compared to that of rats administered other treatment regimens.

4.8 Liver histology and morphometry

The representative histology sections (haematoxylin and eosin staining, 400 X magnifications) on the effect of crude methanolic *F. thonninggi* stem-bark extracts in rats fed a high fructose diet are shown in Figure 4.15. The effect of crude methanolic *F. thonningii* stem-bark extract on the steatosis and inflammation scores in male rats fed a high-fructose diet is are shown in Table 4.8 below.

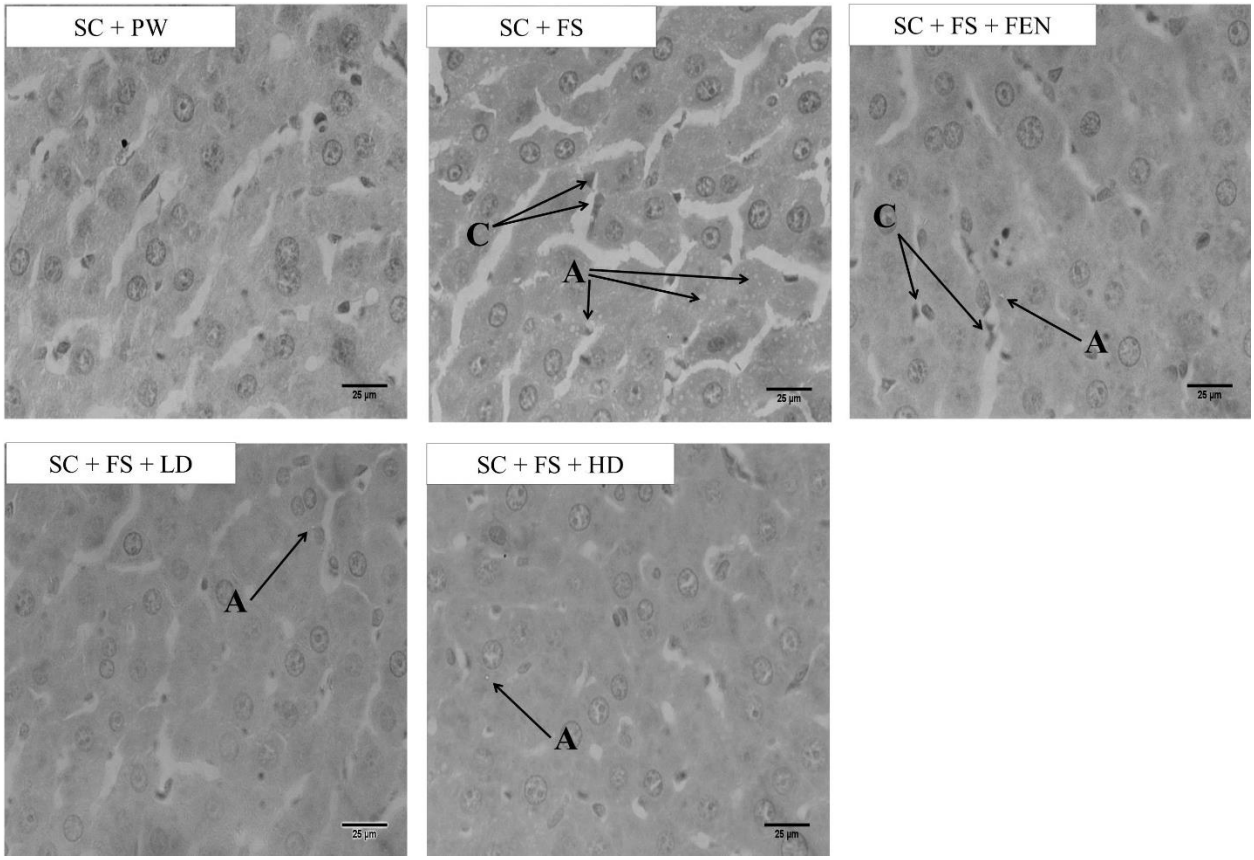


Figure 4.15: Photomicrographs showing male liver histology sections (haematoxylin and eosin staining, 400 X magnification)

Arrows A show microsteatosis and Arrows C shows inflammatory cells; SC+PW = standard rat chow + plain drinking water + plain gelatine cube; SC+FS = standard rat chow + 20% fructose (FS) in drinking water (w/v) + plain gelatine cube; SC+FS+FEN = standard rat chow + 20% FS in drinking water (w/v) + fenofibrate (100 mg/kg body mass/ day); SC+FS+LD = standard rat chow + 20% FS in drinking water (w/v) + low dose *Ficus thonningii* extract (50 mg/kg body mass/ day). SC+ FS+HD = standard rat chow + 20% FS in drinking water (w/v) + high dose *Ficus thonningii* extract (500 mg/kg body mass/ day); Scale bar = 25µm; n = 7-8.

The consumption of the high fructose diet caused microsteatosis and inflammation in the liver while the administration of both the low and high dose crude methanolic *F. thonningii* resulted in microsteatosis with no inflammation.

Table 4.8: Effect of crude methanolic *Ficus thonningii* stem-bark extract on the macro- and micro-morphometry of the liver from male rats fed a high-fructose diet

Parameters	SC+PW	SC+FS	SC+FS+FEN	SC+FS+LD	SC+FS+LD	Significance level
Liver (g)	11.10±1.20[a]	10.50±0.98[a]	13.30±3.09[a]	12.00±3.06[a]	12.00±3.06[a]	ns
%BM	3.35±0.34[ab]	2.56±1.49[a]	4.44±1.23[b]	3.50±0.51[ab]	3.47±0.33[ab]	**
TLr (g/mm)	27.50±3.16[a]	25.50±2.37[a]	34.10±7.90[a]	30.2±7.43[a]	27±4.84[a]	ns
Macrosteatosis	0(0;0)[a]	0(0;0)[a]	0(0;0)[a]	0(0;0)[a]	0(0;0)[a]	ns
Microsteatosis	0(1;0)[a]	1(2;1)[b]	0.5(1;0)[a]	0.5(1;0)[a]	0.5(1;0)[a]	*
Inflammation	0(1;0)[a]	1(1;0)[b]	1(1;0)[b]	0(1:0)[a]	0(1;0)[a]	*

ns = not significant, P> 0.05; *P ≤ 0.05; ** P < 0.01; [ab]Within row means with different superscripts are significantly different at P<0.05). Rats fed a high fructose diet with fenofibrate as an intervention had significantly heavier livers (relative to body mass) compared to the rats fed a control diet (P < 0.01). Rats fed a high fructose diet alone had a significantly higher (P<0.05) micro-steatosis score compared to the rats in other treatment regimens. Rats fed a high fructose diet and those fed a high fructose diet with fenofibrate as an intervention had significantly higher (P<0.05) hepatic inflammation scores than their counterparts administered other treatment regimens. SC+PW = standard rat chow + plain drinking water + plain gelatine cube; SC+FS = standard rat chow + 20% fructose (FS) in drinking water (w/v) + plain gelatine cube; SC+FS+FEN = standard rat chow + 20% FS in drinking water (w/v) + fenofibrate (100 mg/kg body mass/ day); SC+FS+LD = standard rat chow + 20% FS in drinking water (w/v) + low dose *Ficus thonningii* extract (50 mg/kg body mass/ day). SC+ FS+HD = standard rat chow + 20% FS in drinking water (w/v) + high dose *Ficus thonningii* extract (500mg/kg body mass/day). Data presented as mean ± SD, median and IQR; n = 7-8.

Male rats fed a high fructose diet with fenofibrate as an intervention had significantly ($P<0.01$) heavier livers (relative to body mass) compared to the rats fed a high fructose diet. In addition, male rats fed a high fructose diet alone had a significantly higher ($P<0.01$) micro-steatosis score compared to the rats in other treatment regimens. The male rats fed a high fructose diet alone as well as those fed a high fructose diet with fenofibrate as an intervention had higher inflammation scores.

The representative histology sections (haematoxylin and eosin staining, 400 X magnifications) on the effect of crude methanolic *F. thonninggi* stem-bark extracts in female rats fed a high fructose diet are shown in Figure 4.16. The effect of crude methanolic *F. thonningii* stem-bark extract on the steatosis and inflammation scores in male rats fed a high-fructose diet is are shown in Table 4.9 below.

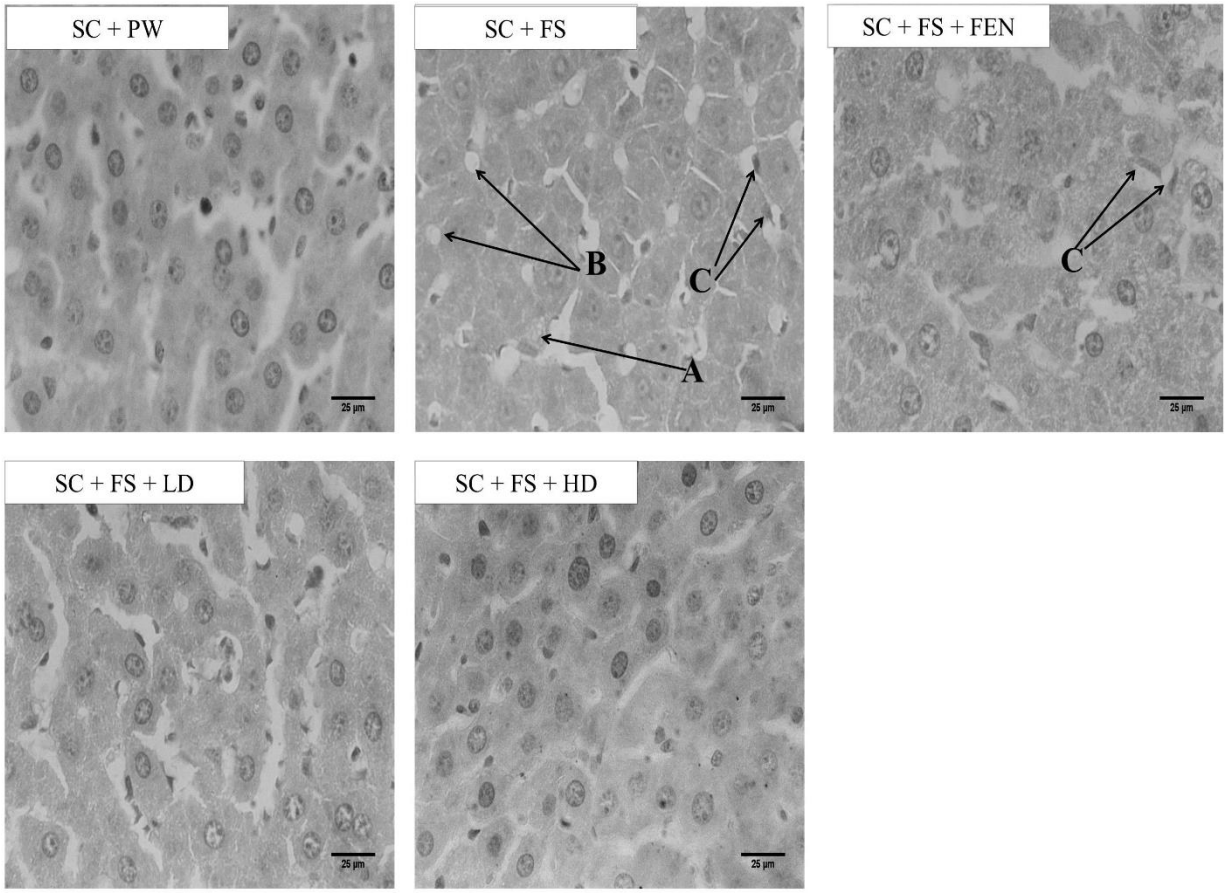


Figure 4.16: Photomicrographs showing female rat liver histology sections (haematoxylin and eosin staining, 400 X magnifications)

Arrow A shows microsteatosis; Arrow B shows macrosteatosis and Arrows C inflammatory cells; SC+PW = standard rat chow + plain drinking water + plain gelatine cube; SC+FS = standard rat chow + 20% fructose (FS) in drinking water (w/v) + plain gelatine cube; SC+FS+FEN = standard rat chow + 20% FS in drinking water (w/v) + fenofibrate (100 mg/kg body mass/ day); SC+FS+LD = standard rat chow + 20% FS in drinking water (w/v) + low dose *Ficus thonningii* extract (50 mg/kg body mass/ day). SC+ FS+HD = standard rat chow + 20% FS in drinking water (w/v) + high dose *Ficus thonningii* extract (500 mg/kg body mass/ day). Scale bar = 25µm; n = 7-8.

Consumption of the high fructose diet caused macro- and micro-steatosis and inflammation in the liver. While fenofibrate prevent the steatosis the low and high dose crude methanolic *F. thonningii* extracts prevented both the high fructose diet induced steatosis and inflammation.

Table 4.9: Effect of crude methanolic *Ficus thonningii* stem-bark extract on the macro- and micro-morphometry of the liver from female rats fed a high-fructose diet

Parameters	SC+PW	SC+FS	SC+FS+FEN	SC+FS+LD	SC+FS+HD	Significance
Liver (g)	7.35± 2.12[a]	8.07± 2.03[a]	9.73± 1.74[a]	8.02±1.39[a]	8.54± 1.58[a]	ns
Liver (% TBM)	3.19± 0.39[a]	3.4± 0.60[a]	4.02± 0.49[b]	3.52± 0.39[ab]	3.50± 0.59[ab]	*
Liver (TL)	18.90±5.80[a]	21.50±5.36[a]	26.00±3.85[a]	21.60 ± 4.22[a]	24.00 ± 3.94[a]	ns
Macro-steatosis	0(0; 0) [a]	2.5(3.0; 2.0) [b]	0(0; 0) [a]	0(0; 0) [a]	0(0; 0) [a]	***
Micro-steatosis	0(1; 0) [a]	2(3;1) [b]	1(2;1) [a]	0.5(1; 0) [a]	0(0; 0) [a]	*
Inflammation	0(0; 0) [a]	1(1; 0) [b]	0(1; 0) [ab]	0(0: 0) [a]	0(0; 0) [a]	**

ns = not significant, P> 0.05; *P < 0.05. ** P < 0.01; [ab]Within row means with different superscripts are significantly different at P<0.05. Rats fed a high fructose diet with fenofibrate as an intervention had significantly heavier livers (relative to body mass) compared to the rats fed a control diet (P < 0.01). The rats fed a high fructose diet had significantly higher steatosis scores P<0.05 compared to rats fed a control diet or a high fructose diet with fenofibrate or *F. thonningii* extract (high and low dose) as interventions. SC+PW = standard rat chow + plain drinking water + plain gelatine cube; SC+FS = standard rat chow + 20% fructose (FS) in drinking water (w/v) + plain gelatine cube; SC+FS+FEN = standard rat chow + 20% FS in drinking water (w/v) + fenofibrate (100 mg/kg body mass/ day); SC+FS+LD = standard rat chow + 20% FS in drinking water (w/v) + low dose *F. thonningii* extract (50mg/kg body mass/ day). SC+ FS+HD = standard rat chow + 20% FS in drinking water (w/v) + high dose Ficus thonningii extract (500mg/kg body mass/ day). Data presented as mean ± SD, median and IQR; n = 7-8.

Female rats fed a high fructose diet with fenofibrate as an intervention had significantly ($P < 0.01$) heavier livers (relative to body mass) compared to the rats fed a high fructose diet. While the rats fed a high fructose diet had significantly higher ($P<0.01$) steatosis scores compared to the rats administered other treatments regimens, there was no significant difference ($P>0.05$) in the scores of inflammation between rats fed a high fructose diet and the rats fed a high fructose diet with fenofibrate as an intervention but rats administered the low and high dose *F. thonningii* stem-bark extract as an intervention did not show hepatic inflammation.

4.9 Kidney histology and morphometry

The representative histology sections (haematoxylin and eosin staining, 400 X magnifications) on the effect of the crude methanolic *F. thonninggi* stem-bark extracts on male rat kidneys are shown in Figure 4.17 and Table 4.10 shows the of the extract on kidney macro- and micro-morphometry.

Treatment regimens had no effect on kidney micro-morphometry.

There were no significant differences ($P> 0.05$) in the kidney mass (relative and absolute) corpuscular area, glomerular tuft area and urinary space areas of kidneys from male rats across treatment regimens.

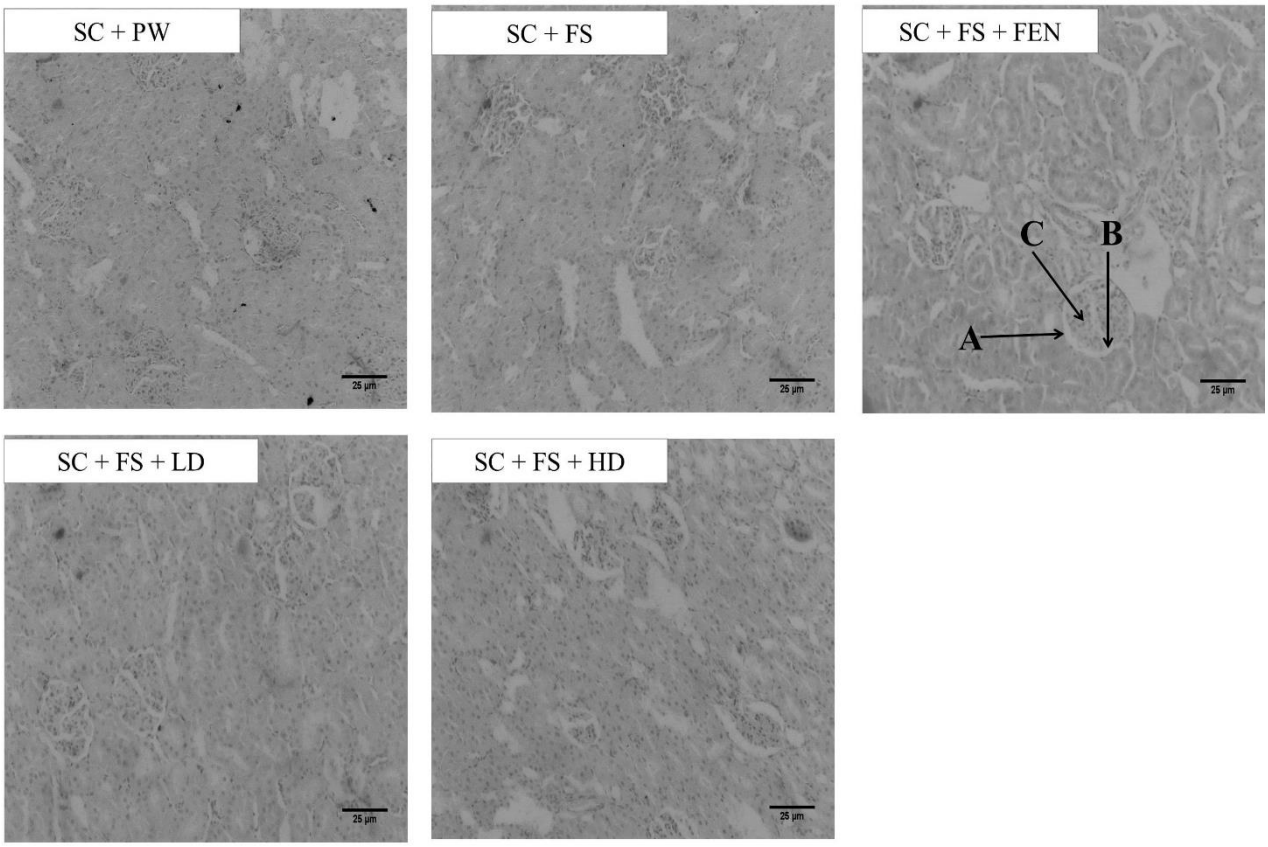


Figure 4.17: Photomicrographs showing male rat kidney histology sections (haematoxylin and eosin staining, 400 X magnifications)

Arrow A shows glomerular corpuscle, Arrow B shows the urinary space and Arrow C shows the glomerulus; SC+PW = standard rat chow + plain drinking water + plain gelatine cube; SC+FS = standard rat chow + 20% fructose (FS) in drinking water (w/v) + plain gelatine cube; SC+FS+FEN = standard rat chow + 20% FS in drinking water (w/v) + fenofibrate (100 mg/kg body mass/ day); SC+FS+LD = standard rat chow + 20% FS in drinking water (w/v) + low dose *Ficus thonningii* extract (50 mg/kg body mass/ day). SC+ FS+HD = standard rat chow + 20% FS in drinking water (w/v) + high dose *Ficus thonningii* extract (500 mg/kg body mass/ day). Scale bar = 25µm; n = 7-8.

Table 4.10: Effect of crude methanolic *Ficus thonningii* stem-bark extract on the macro- and micro-morphometry of the kidneys from male rats fed a high-fructose diet

Parameters	SC+PW	SC+FS	SC+FS+FEN	SC+FS+LD	SC+FS+HD	Significance level
Kidneys (g)	2.36±0.23[a]	2.26±0.19[a]	2.41±0.28[a]	2.42±0.24[a]	2.22±0.23[a]	ns
%BM	0.71±0.07[a]	0.72±0.05[a]	0.80±0.12[a]	0.71±0.02[a]	0.73±0.06[a]	ns
TLr (g/mm)	5.85±0.64[a]	5.69±0.43[a]	6.17±0.69[a]	6.08±0.68[a]	5.63±0.69[a]	ns
Corpuscular area (µm²)	9611.00±852.90[a]	8136.00±1974.00[a]	9317.00±2655.00[a]	10419.00±804.70[a]	9537.00±2626.00[a]	ns
Glomerular tuft area (µm²)	9696.00±753.00[a]	9951.00±2220.00[a]	12500.00±3406.00[a]	13629±757.00[a]	9114.00±2604.00	ns
Urinary space area (µm²)	14.43±4.27 [a]	13.16±1.10 [a]	14.00±11.27 [a]	12.41±3.75 [a]	14.59±5.89 [a]	ns

ns = not significant, P> 0.05. Kidney mass, corpuscular area, glomerular tuft area and urinary space area of the rats were similar across treatment regimens. SC+PW = standard rat chow + plain drinking water + plain gelatine cube; SC+FS = standard rat chow + 20% fructose (FS) in drinking water (w/v) + plain gelatine cube; SC+FS+FEN = standard rat chow + 20% FS in drinking water (w/v) + fenofibrate (100 mg/kg body mass/ day); SC+FS+LD = standard rat chow + 20% FS in drinking water (w/v) + low dose *F. thonningii* extract (50 mg/kg body mass/ day). SC+ FS+HD = standard rat chow + 20% FS in drinking water (w/v) + high dose *F. thonningii* extract (500 mg/kg body mass/ day). Data presented as mean ± SD; n = 7-8.

The representative histology sections (haematoxylin and eosin staining, 400 X magnifications) on the effect of the crude methanolic *F. thonningii* stem-bark extracts on female rat kidneys are shown in Figure 4.18 and Table 4.11 shows the effect of the extract on kidney macro- and micro-morphometry.

Treatment regimens had no effect on kidney micro-morphometry.

There were no significant differences ($P>0.05$) in the kidney mass (relative and absolute) and corpuscular area, glomerular tuft area and urinary space area of kidneys from female rats across treatment regimens.

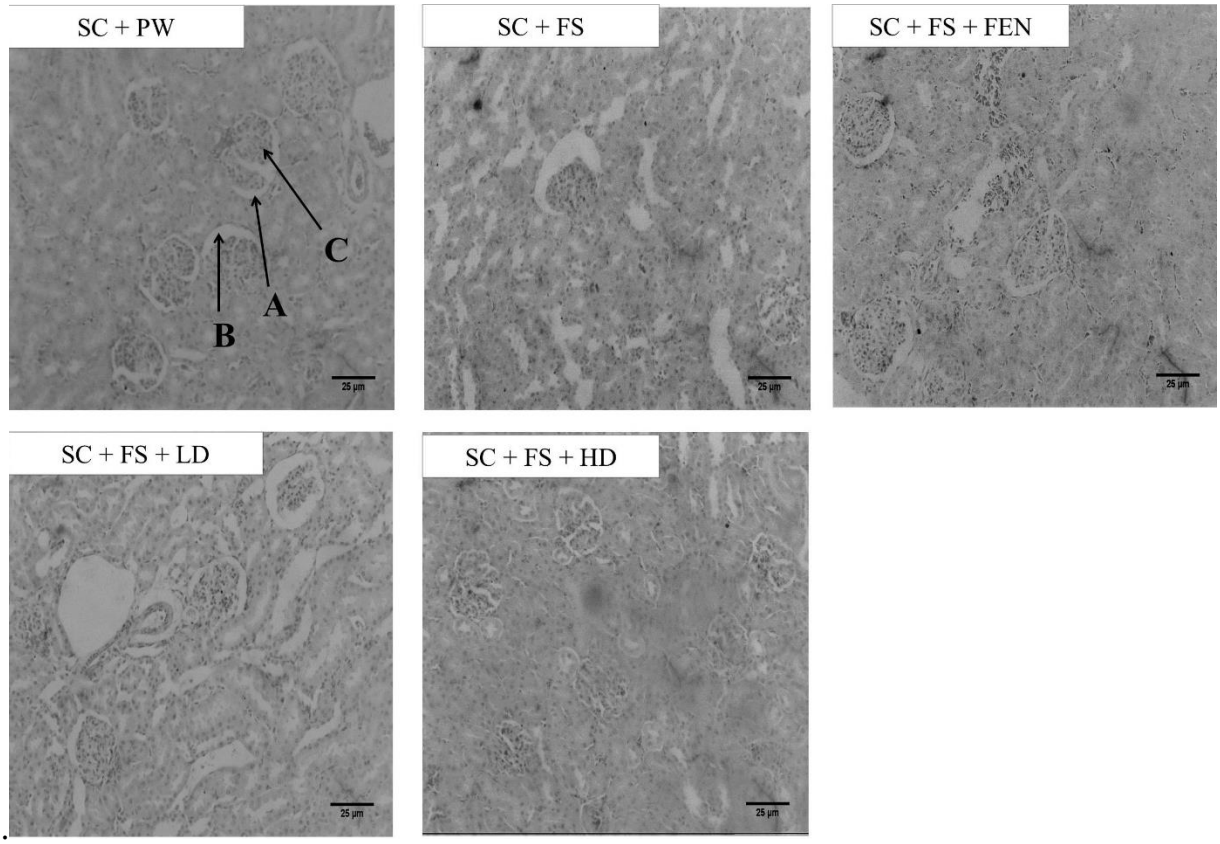


Figure 4.18: photomicrographs showing the female kidney histology sections (haematoxylin and eosin staining, 400 X magnifications)

Arrow A shows glomerular corpuscle, Arrow B shows the urinary space and Arrow C shows the glomerulus; SC+PW = standard rat chow + plain drinking water + plain gelatine cube; SC+FS = standard rat chow + 20% fructose (FS) in drinking water (w/v) + plain gelatine cube; SC+FS+FEN = standard rat chow + 20% FS in drinking water (w/v) + fenofibrate (100 mg/kg body mass/ day); SC+FS+LD = standard rat chow + 20% FS in drinking water (w/v) + low dose *Ficus thonningii* extract (50 mg/kg body mass/ day). SC+ FS+HD = standard rat chow + 20% FS in drinking water (w/v) + high dose *Ficus thonningii* extract (500 mg/kg body mass/ day). Scale bar = 25µm; n = 7-8.

Table 4.11: Effect of crude methanolic *Ficus thonningii* stem-bark extract on the macro- and micro-morphometry of the kidneys from female rats fed a high-fructose diet

Parameters	SC+PW	SC+FS	SC+FS+FEN	SC+FS+LD	SC+FS+HD	Significance level
Kidneys (g)	1,647±0.31[a]	1,672±0.10[a]	1,857±0.14[a]	1,636±0.26[a]	1,787±0.17[a]	ns
%BM	0.73±0.05[a]	0.71±0.04[a]	0.77±0.01[a]	0.72±0.09[a]	0.73±0.05[a]	ns
TLr (g/mm)	4.29±0.87[a]	4.46±0.29[a]	4.98±0.26[a]	4.40±0.76[a]	5.03±0.43[a]	ns
Corpuscular area (µm²)	10855.00±1034.00[a]	8820.00±1834.00[a]	10745.00±447.5[a]	8369.00±1189.00[a]	8744.00±1357.00[a]	ns
Glomerular tuft area (µm²)	15549.00±2312.00[a]	14191.00±3916.00[a]	13269.00±2941.00[a]	13508.00±1714.00[a]	13681.00±1372.00[a]	ns
Urinary space area (µm²)	12.29±0.63[a]	14.98±15.89[a]	13.04±4.10[a]	14.29±3.38[a]	13.61±0.87[a]	ns

ns = not significant, P> 0.05. Kidney mass, corpuscular area, glomerular tuft area and urinary space area were similar across treatment groups. SC+PW = standard rat chow + plain drinking water + plain gelatine cube; SC+FS = standard rat chow + 20 % fructose (FS) in drinking water (w/v) + plain gelatine cube; SC+FS+FEN = standard rat chow + 20% FS in drinking water (w/v) + fenofibrate (100 mg/kg body mass/ day); SC+FS+LD = standard rat chow + 20% FS in drinking water (w/v) + low dose *Ficus thonningii* extract (50 mg/kg body mass/ day). SC+ FS+HD = standard rat chow + 20% FS in drinking water (w/v) + high dose *Ficus thonningii* extract (500 mg/kg body mass/ day). Data presented as mean ± SD; n = 7-8.

4.10 Surrogate markers of general health.

The effects of crude methanolic *F.thonningii* extracts on plasma creatinine, blood urea nitrogen, cholesterol concentration and ALT and ALP activities of male rats are shown in Table 4.12 below.

There were no significant differences in the surrogate markers of the general health profile of the male rats across treatment regimens.

Table 4.12: Effects of crude methanolic *Ficus thonningii* stem-bark extracts on plasma creatinine, blood urea nitrogen, cholesterol concentration and ALT and ALP activities of male rats fed a high-fructose diet

Parameter	SC+PW	SC+FS	SC+FS+FEN	SC+FS+LD	SC+FS+HD	Significance level
Creatinine (mmol/L)	21.50±4.81[a]	19.70±3.60[a]	18.40±1.17[a]	23.50±4.17[a]	20.10±4.19[a]	ns
BUN (mmol/L)	6.62±0.88[a]	6.43±1.79[a]	6.47±1.28[a]	7.03±2.11[a]	16.30±2.60[a]	ns
ALT (U/L)	85.10±26.00[a]	74.30±38.90[a]	62.00±20.00[a]	71.50±33.40[a]	77.40±50.40[a]	ns
ALP (U/L)	181.00±78.10[a]	227.00±41.90[a]	234.00±66.60[a]	219.00±69.90[a]	224.00±45.90[a]	ns
Cholesterol (mmol/L)	1.40±0.35[a]	1.59±0.09[a]	1.58±0.22[a]	1.62±0.18[a]	1.64±0.15[a]	ns

ns = not significant, P> 0.05. There were no significant differences (P> 0.05) in the plasma creatinine, blood urea nitrogen, cholesterol concentration and ALT and ALP activities of male rats across treatment regimens. SC+PW = standard rat chow + plain drinking water + plain gelatine cube; SC+FS = standard rat chow + 20% fructose (FS) in drinking water (w/v) + plain gelatine cube; SC+FS+FEN = standard rat chow + 20% FS in drinking water (w/v) + fenofibrate (100 mg/kg body mass/ day); SC+FS+LD = standard rat chow + 20% FS in drinking water (w/v) + low dose *Ficus thonningii* extract (50 mg/kg body mass/ day). SC+ FS+HD = standard rat chow + 20% FS in drinking water (w/v) + high dose *Ficus thonningii* extract (500 mg/kg body mass/ day); ALP = Alanine phosphatase; ALT = Alanine aminotransferase; BUN = Blood urea nitrogen. Data presented as mean ± SD; n = 7-8.

The effects of crude methanolic *F.thonningii* extracts on plasma creatinine, blood urea nitrogen, cholesterol concentration and ALT and ALP activities of female rats are shown in Table 4.13 below.

There were no significant differences in the surrogate markers of the general health profile the female rats across treatment regimens.

Table 4.13: Effects of crude methanolic *Ficus thonningii* stem-bark extracts on surrogate markers of general health of female rats fed a high-fructose diet

Parameter	SC+PW	SC+FS	SC+FS+FEN	SC+FS+LD	SC+FS+HD	Significance level
Creatinine (mmol/L)	32.30±9.20[a]	21.40±4.64[a]	21.70±4.48[a]	26.30±9.70[a]	22.3±5.14[a]	ns
BUN (mmol/L)	6.28±1.42[a]	5.55±1.71[a]	6.84±1.18[a]	6.45±0.71[a]	5.35±1.64[a]	ns
ALT (U/L)	55.40±16.10[a]	55.40±16.10[a]	71.20±62.30[a]	49.40±12.70[a]	51.3±19.80[a]	ns
ALP (U/L)	159.00±82.90[a]	183.00±66.70[a]	169.00±49.00[a]	153.00±35.9[a]	172.00±40.80[a]	ns
Cholesterol (mmol/L)	1.59±0.54[a]	1.78±0.05[a]	1.74±0.32[a]	1.75±0.17[a]	1.87±0.29[a]	ns

ns = not significant, P> 0.05. There were no significant differences (P> 0.05) in the plasma creatinine, blood urea nitrogen, cholesterol concentration and ALT and ALP activities of female rats across treatment regimens SC+PW = standard rat chow + plain drinking water + plain gelatine cube; SC+FS = standard rat chow + 20% fructose (FS) in drinking water (w/v) + plain gelatine cube; SC+FS+FEN = standard rat chow + 20% FS in drinking water (w/v) + fenofibrate (100 mg/kg body mass/ day); SC+FS+LD = standard rat chow + 20% FS in drinking water (w/v) + low dose *Ficus thonningii* extract (50 mg/kg body mass/ day). SC+ FS+HD = standard rat chow + 20% FS in drinking water (w/v) + high dose *Ficus thonningii* extract (500 mg/kg body mass/ day); ALP = Alanine phosphatase; ALT = Alanine aminotransferase; BUN = Blood urea nitrogen. Data presented as mean ± SD; n = 7-8.

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4.11 Plasma metabolite concentration and HOMA-IR index

The effects of the methanolic stem bark extracts of *F. thonningii* on the plasma glucose, cholesterol, triglyceride and insulin concentration as well as the HOMA-IR of the male rats fed a high fructose diet are shown in Table 4.14 below.

There were no significant differences ($P>0.05$) in triglyceride, glucose and insulin concentrations and HOMA-IR index of male rats across treatment regimens.

Table 4.14: Effects of crude methanolic *Ficus thonningii* stem-bark extracts on blood triglyceride, glucose and insulin concentration and HOMA-IR index of male rats fed a high-fructose diet

Parameter	SC+PW	SC+FS	SC+FS+FEN	SC+FS+LD	SC+FS+HD	Significance level
Triglyceride (mmol/L)	1.38±0.61[a]	1.9±0.93[a]	1.51±0.55[a]	1.39±036[a]	1.39±0.24[a]	ns
Glucose (mmol/L)	4.54±0.77[a]	4.26±0.64[a]	4.49±0.54[a]	3.93±0.41[a]	4.26±0.57[a]	ns
Insulin (μg/L)	38.68±29.2[a]	37.02±20.9[a]	31.42±18.99[a]	29.02±21.7[a]	29.59±13.68[a]	ns
HOMA-IR	7.73±5.60[a]	6.82±3.69[a]	5.99±3.57[a]	5.21±4.13[a]	5.40±2.61[a]	ns

ns = not significant, P> 0.05. The male rats' mean triglyceride, glucose and insulin concentrations and HOMA-IR indices were similar across treatment regimens. SC+PW = standard rat chow + plain drinking water + plain gelatine cube; SC+FS = standard rat chow + 20% fructose (FS) in drinking water (w/v) + plain gelatine cube; SC+FS+FEN = standard rat chow + 20% FS in drinking water (w/v) + fenofibrate (100 mg/kg body mass/ day); SC+FS+LD = standard rat chow + 20% FS in drinking water (w/v) + low dose *F. thonningii* extract (50 mg/kg body mass/ day). SC+ FS+HD = standard rat chow + 20% FS in drinking water (w/v) + high dose *F. thonningii* extract (500mg/kg body mass/day). Data presented as mean ± SD; n = 7-8.

The effects of the methanolic stem bark extracts of *F. thonningii* on the plasma glucose, cholesterol, triglyceride and insulin concentration as well as the HOMA-IR of the female rats fed a high fructose diet are shown in Table 4.15 below.

While blood glucose and insulin concentration and HOMA-IR index of the rats were similar ($P>0.05$) across treatment regimens, rats fed a high fructose diet alone had significantly higher blood triglyceride concentration ($P<0.05$) compared to that of rats fed a control diet**.**

Table 4.15: Effects of crude methanolic *Ficus thonningii* stem-bark extracts on blood triglyceride, glucose and insulin concentration and HOMA-IR index of female rats fed a high-fructose diet

Parameter	SC+PW	SC+FS	SC+FS+FEN	SC+FS+LD	SC+FS+HD	Significance level
Triglyceride (mmol/L)	1.48±027[a]	2.04±0.33[b]	1.60±0.25[ab]	1.60±0.35[ab]	1.43±0.34[ac]	*
Glucose (mmol/L)	4.26±0.64[a]	4.24±0.48[a]	4.49±0.54[a]	3.94±0.46[a]	4.34±0.72[a]	ns
Insulin (µg/L)	27.85±13.47[a]	28.01±19.27[a]	24.67±18.65[a]	22.71±15.77[a]	37.57±20.6[a]	ns
HOMA-IR	5.07±3.34[a]	5.09±3.32[a]	4.85±3.51[a]	4.52±3.25[a]	7.09±3.35[a]	ns

ns = not significant, P> 0.05; * P ≤ 0.05; [abc]Within row means with different superscripts are significantly different (P< 0.05). Rats fed a high fructose diet alone had significantly higher blood triglyceride concentration P (≤ 0.05) compared to that of rats fed a control diet**.** Rats fed a high fructose diet with a high dose of *F.thonningii* as an intervention had significantly lower blood triglyceride concentration P (<0.05) compared to that of rats fed a high fructose diet alone**..** SC+PW = standard rat chow + plain drinking water + plain gelatine cube; SC+FS = standard rat chow + 20 % fructose (FS) in drinking water (w/v) + plain gelatine cube; SC+FS+FEN = standard rat chow + 20% FS in drinking water (w/v) + fenofibrate (100 mg/kg body mass/ day); SC+FS+LD = standard rat chow + 20% FS in drinking water (w/v) + low dose *Ficus thonningii* extract (50 mg/kg body mass/ day). SC+ FS+HD = standard rat chow + 20% FS in drinking water (w/v) + high dose *Ficus thonningii* extract (500 mg/kg body mass/ day). Data presented as mean ± SD; n = 7-8.

CHAPTER 5: DISCUSSION

5.1 Phytochemical constituents of *F. thonningii* stem-bark extracts

Qualitative phytochemical screening of *F. thonningii* stem-bark extract revealed the presence of terpenoids, saponnins, flavonoids and tannins (Table 4.1). Studies done previously on the phytochemistry of *F. thonningii* stem-bark and leaves reported the presence of the same phytochemicals (Ndukwe et al., 2007; Usman & Usman, 2009) thus findings from the current study are in conformity with those reported in earlier studies. Terpenoids, saponnins, flavonoids and tannins have been hypothesised to give *F. thonningii* stem-bark extracts their antioxidant, lypolytic and hypoglycaemic properties (Aba & Asuzu, 2018). These properties make *F. thonningii* stem-bark extracts potential prophylactic agents in the prevention of the development of metabolic derangements caused by obesogenic diets and obesity in growing children. In the current study it can therefore be speculated that some of the "protective" effects of the stem/bark extract against some of the diet-induced metabolic effects are likely to be due to the presence of these health beneficial bioactive phytochemicals.

5.2 Effect of *F. thonningii* stem-bark extracts on tolerance to a glucose load

In the present study, there were no significant differences in the tolerance to an oral glucose challenge by both male and female rats across treatment regimens (Figures 4.4 and 4.5). Importantly the rats' plasma insulin concentration and HOMA-IR were also similar across treatment regimens. These similarities suggest that the high fructose diet, fenofibrate or *F. thonningii* extracts did not cause hyperglycaemia, neither did they impair glucose tolerance nor cause the development of insulin resistance in both male and female rats. Impaired glucose tolerance has been observed in 21-day old rats that were fed a 20% fructose solution for 6 weeks (Dupas et al., 2017). Bocarsly et al. (2010) also observed glucose intolerance and hyperglycaemia in adolescent Sprague Dawley rats following the consumption of 10% fructose in drinking water for 8 weeks. Findings in the current study contradict with the observations by Dupas et al. (2017) and Bocarsly et al. (2010). The observed contradiction is difficult to explain. In the case of the findings by Dupas et al. (2017) the current study used similar-aged rats and a similar fructose solution dose and for a longer time, thus one would have expected the development of glucose intolerance and hyperglycaemia. Regarding the findings reported by Bocarsly et al. (2010), in view of the fact that the current study used a higher fructose concentration, one would have also expected the development of glucose intolerance and hyperglycaemia. In a study on the effect of fructose ingestion protocols on markers of metabolic syndrome in Wistar rats, de Moura et al (2009) observed that fructose incorporated in the feed rather than in drinking water is a more effective way of inducing

markers of the metabolic syndrome including glucose intolerance and insulin resistance. Additionally high fructose feeding was shown to be more effective at producing signs of the metabolic syndrome in adult than in young rats (Ghezzi et al., 2012). In the current study it can be speculated that failure of a high-fructose diet to induce hyperglycaemia and insulin resistance in rats could be attributed to the differences in the rat strains and the sources of fructose utilised.

F. thonningii has been shown to possess hypoglycaemic effects thus explaining its traditional use in the treatment and management of diabetes mellitus. Musabayane *et al*. (2012) and Gondwe *et al*. (2007) showed that the oral administration of *F. thinningii* stem-bark extracts at 500mg/kg body mass/day and 250mg/kg/ body mass/day for 5-weeks, reduced fasting blood glucose concentration in streptozotocin-induced diabetic rats following an oral glucose challenge. In the current study, fructose did not induce hyperglycaemia in rats hence the potential hypoglycaemic effects of *F. thonningii* were not manifest.

While treatment with fenofibrate was shown to improve glycaemic control and insulin resistance in type II diabetic patients (Damci et al., 2003), Liu *et al*. (2011) observed that treatment with fenofibrate disrupted pancreatic beta cell function thus impairing glucose-stimulated insulin secretion in obese Wistar rats. Results of the current study neither agree nor disagree with the findings of Damci *et al* (2003) and those by Liu *et al*. (2011) since no hyperglycaemia was observed.

5.3 Effect of *F. thonningii* stem-bark extract on growth and long bone indices

5.3.1 Body mass

In the present study, a high fructose diet alone and or with fenofibrate or *Ficus thonningii* stem-bark extract as an intervention had no effect on growth as measured by body mass in both male and female rats compared to rats on a control diet suggesting that the interventions did not compromise growth as measured by body mass. However, body mass is an unreliable measure of growth because it can be affected by various factors such as obesity (Owen et al., 2005), hydration state, prandial state and visceral organ size (Stookey, 2016). Several studies in adult animals reported an increase in body mass of adult rats following high fructose feeding (Pektaş et al., 2015; Toop & Gentili, 2016). In the current study, there were no differences observed in the terminal body masses and empty carcass masses of male and

female rats across treatment regimens. It is important to point out that those previous studies utilised adult rats whereas the current study weanling rat pups were used. Weanling rats are still growing and are most likely to channel the "extra" calories to supporting growth and development unlike adult rats that would accrete the excess calories as adipose tissue. Interestingly results of the current study agree with reports by Huang et al. (2004) and Grau et al. (2018), who did not observe any significant differences in body mass of adolescent Sprague Dawley rats following the consumption of dietary fructose (60% in drinking water) for 8 and 16 weeks, respectively. Although in previous studies *F. thonningii* stem-bark extracts have been shown to increase body mass of rats (Badiora et al., 2016; Gondwe, 2007), findings of the present study show that *F. thonningii* did not promote growth as measured by terminal body mass and empty carcass mass in growing rats but across treatment regimens, the rats grew significantly showing that the extract did not compromise growth.

5.3.2 Effect of *F. thonningii* stem-bark extracts on long bone parameters

The long bone (tibia and femora) parameters have been used in several studies as more accurate determinants of growth performance since they respond to growth hormone in a dose-dependent manner (Orwoll et al., 2009). In the current study feeding male rats with a high fructose diet alone or with fenofibrate or *F. thonningii* as interventions did not affect the rats' tibiae masses and lengths (Table 4.2). The high fructose diet had no effect on the tibia and femora masses, lengths and density of the female rats. However, the high dose crude methanolic *F. thonningii* stem-bark extract decreased tibiae length and the low dose crude methanolic *F. thonningii* stem-bark extract reduced the femora density in female rats. This suggests that the high and low doses of *Ficus thonningii* extract compromised bone length and density respectively. Taken together, these findings infer that *Ficus thonningii* extracts compromised the growth performance and bone strength of the female rats. The mechanism behind these findings however need further investigation. In male rats, the consumption of a high fructose diet decreased femora density that fenofibrate and the high dose crude methanolic *F. thonningii* extract failed to attenuate. However, the low dose crude methanolic *F. thonningii* stem-bark extract attenuated the high fructose diet induced decrease in femora density in male rats (Table 4.2). These findings suggest that the high fructose diet compromised femora density of male rats. It can therefore be inferred that high fructose diet exhibited sexual dimorphism on its impact on bones. These findings are at variance with those of Shi et al. (2017) who reported that treatment with fenofibrate caused a decrease in bone mass in high-fat high-fructose induced diabetic mice. Lucas et al. (2017) observed that the

consumption of fructose alone or in combination with fenofibrate had deleterious effects on long bone density; findings that are in tandem with the observed effects of the high fructose diet on femora density in male rats in the current study. Our study demonstrated that the high fructose diet has detrimental effects on femora of male rats while the low dose crude methanolic *F. thonningii* stem-bark extract protects femora against the diet-induced loss of density. The mechanism behind these findings, however, need further investigation.

5.4 Effect of *F. thonningii* stem-bark extracts on viscera morphometry

The GIT organ morphometry is used to indicate gut health (Guinane & Cotter, 2013). Several chemical compounds have been reported to affect the development of parts of the GIT (Laparra & Sanz, 2010). Various phytochemicals have also been reported to cause changes in the function and structure of the GIT (Laparra & Sanz, 2010) . In the current study, in both male and female rats, the consumption of a high fructose diet and or a high fructose diet combined with fenofibrate or high or low dose of crude methanolic *F. thonningii* extract as interventions did not have any effect on the mass of the stomach and caeca of both male and female rats. However, in male rats, a high fructose diet with a high dose of crude methanolic *F. thonningii* stem-bark extract as an intervention increased large intestine mass (absolute) and length. This could be attributed to the high fibre content in *F. thonningii* stem-bark extract (Badiora et al., 2016). High dietary fibre content has been reported to cause an increase in the length and weight of the large intestines in rats (Zhao et al., 1995). While a high fructose diet with a high dose of crude methanolic *F. thonningii* extract increased large intestine mass and length in male rats, a high fructose diet with a low dose of crude methanolic *F. thonningii* extracts increased the length and small intestine mass (relative to body mass and tibia length) in female rats. The findings from the current study show that feeding a high fructose diet with a high or low dose of *F.thonningii* stem bark extracts in growing Sprague-Dawley rats shows sexual dimorphism in regard to dietary fructose's effects on the macro-morphometry of the small and large intestines. The mechanism behind these findings however need further investigation.

There is a strong correlation between organ mass and absolute body mass (Bailey et al., 2004) hence relative organ mass is more reliable in comparison to absolute organs masses of rats in different treatment groups as it accounts for the differences in body mass (Bailey et al., 2004). The consumption of 20% fructose by adult Sprague Dawley rats for 8 weeks induced cardiac and renal tissue hypertrophy (Saleh et al., 2017). In addition studies using human and animal models have shown increases in liver and visceral fat pad masses following the consumption

of a high fructose diet (Crescenzo et al., 2012). In the current study, however, the consumption of a high-fructose diet for 8 weeks by growing Sprague Dawley rats did not affect the absolute and relative masses of the heart, kidney, liver, pancreas, visceral and epididymal fat pad in both male and female rats when compared to those from counterparts fed the control diet. Studies have shown that the consumption of a high-fructose diet with fenofibrate as an intervention causes hepatomegaly (Gasa, 2012). In the current study the oral administration of fenofibrate as an intervention in high fructose diet fed Sprague Dawley rats caused a significant increase in liver mass (relative to body mass) in both male and female rats suggesting the development of hepatomegaly and in tandem with the reports by Gasa (2012).

5.5 Effect of *F. thonningii* stem-bark extracts on NAFLD parameters

In the current study in male rats, the consumption of a high fructose diet caused hepatic micro-steatosis and inflammation (Table 4.8). The high fructose diet-induced micro-steatosis was prevented by the oral administration fenofibrate and or both the low and high dose *F. thonningii* stem-bark extract. The observed hepatic inflammation was only prevented by the *F. thonningii* extracts (Table 4.8). In female rats the high fructose diet caused macro- and micro-steatosis and inflammation which were mitigated by fenofibrate and both the low and high dose *F. thonningii* extracts (table 4.9). These results clearly demonstrate that consumption of high fructose diet compromises liver health in both sexes and is in agreement with the findings by (Choi et al., 2017) who reported that diets high in fructose promote liver steatosis in rats. From the finding of the current study it can be inferred that while both fenofibrate and the *F. thonnigii* extracts could be used to prevent steatosis; fenofibrate cannot be used to protect against diet-induced hepatic inflammation. Importantly, results from the current suggest that crude methanolic *F. thonningii* stem-bark extracts can be used as a prophylaxis against diet-induced hepatic steatosis and inflammation in growing children fed obesogenic diets. In the current study the consumption of a high fructose diet alone and or intervening with either fenofibrate or *Ficus thonningii* stem-bark extract had no effect on kidney morphometry in both male and female rats compared to rats. This observation suggests that the interventions did not compromise renal function.

5.6 Effect of F. thonningii stem-bark extracts on haematocrit and erythrocyte fragility

Ahur et al. (2013) reported that *F. thonningii* leaf extracts improved red blood cell count in acetaminophen-treated rats and Dangarembizi et al. (2014) also reported that *F. thonningii*

leaf extracts of caused a decrease in the PCV in rat pups. In addition, Coker et al. (2009) reported that aqueous *F. thonningii* leaf extracts had no deleterious effects on the rats' haematocrit. Results of the current study corroborate with finding by Coker et al. (2009) wherein the consumption of a high-fructose diet with *F. thonningii* extract as an intervention had no significant effect (compared to control) on the packed cell volume of both the male and female rats. It is noteworthy that a high-fructose diet alone or a high fructose diet with fenofibrate as an intervention also did not compromise the rats' haematocrit These results suggest that the high-fructose diet alone and or with fenofibrate and or with *F. thonningii* extracts, as intervention, did not alter the rats' haematocrit. This finding could be interpreted to mean that the high-fructose alone and or with fenofibrate and or with *F. thonningii* extracts did not cause the haemolysis of erythrocytes.

The erythrocyte membrane fluidity is influenced by several factors including plasma lipid content (Ferreri et al., 2016). Dietary fatty acid composition affects plasma cholesterol and triglyceride content in rats leading to altered fatty acid composition of erythrocyte membranes and their fluidity (Giardina et al., 2018). This results in significantly increased susceptibility of hyperlipidaemic erythrocytes of the rats to osmotic fragility (Kalmath et al., 2017). Sengupta and Ghosh (2011) reported that membrane fluidity in rat erythrocytes diminishes during hyperlipidaemia but can be attenuated by administration of *F. thonningii* extracts (Ahur & Adenkola, 2013) In the present study there were no significant differences in the osmotic fragility of erythrocytes of rats across treatment regimens. These findings suggest that a high-fructose diet, fenofibrate and or *F. thonningii* extracts did not cause any deleterious effects on the rats' erythrocyte membranes. These findings can be taken to mean that fenofibrate and or *F. thonningii* extracts may be used without the fear of any negative effects on erythrocyte membrane fragility.

5.7 Effect of *F. thonningii* stem-bark extracts on markers of health

The liver is a major organ involved in drug biotransformation, nutrient processing and glycogen and lipid storage (Alamri, 2018; Chiang, 2014). The 'gold standard' in assessing liver function is a liver biopsy (Fallatah, 2014). However, surrogate biomarkers such as serum activities of ALP and ALT can also be used to assess liver function (Gowda et al., 2009). Hepatocytes are prone to damage because of their exposure to toxins (Jain & Kaplowitz, 2010) and damage to liver cells causes leakage of liver enzymes; AST, ALT and ALP into the blood hence the plasma activities of these enzymes are used as surrogate markers of liver function. However, plasma ALT and AST activity are deemed better surrogate markers for

liver damage as they are more specific to the liver since plasma ALP activity is also influenced by bone growth and pregnancy (Li et al., 2014). In the present study I observed that a high-fructose diet alone or a high-fructose diet with either fenofibrate or *F. thonningii* extracts as interventions in both male and female rats did not cause an increase in plasma activities of ALP or ALT (Tables 4.10 and 4.11, respectively) suggesting that the different treatment regiments did not cause liver damage.

The gold standard for determining kidney function is the glomerular filtration rate (GFR) (Levey and Inker, 2016). However surrogate biomarkers of kidney function such as plasma creatinine and blood urea nitrogen (BUN) concentration can also be used to determine kidney health since elevated plasma levels of the two parameters indicate impaired glomerular filtration (Edelstein, 2008). According to Abdel-Kawi et al. (2016), the consumption of fructose is one of the factors that contributes to the metabolic syndrome and consequently to chronic kidney disease. Yang et al. (2014) observed an increase in plasma concentrations of creatinine and BUN adult albino rats following the consumption of fructose (10% w/v) for 5 weeks. In the current study fructose consumption alone or either fenofibrate and or *F. thonningii* extract as interventions did not significantly affect plasma creatinine and BUN concentrations (Tables 4.10 and 4.11). These findings are in agreement with the findings of Badiora et al. (2016) wherein the consumption of *F. thonningii* stem bark extract at dosages of up to 1000mg/kg body mass did not have any adverse effects on kidney function of adult rats. *F. thonningii* extracts have been shown to cause reno-protective effects by lowering plasma BUN and creatinine concentrations in streptozotocin-induced diabetic rats (Musabayane et al., 2007). This might be the reason why in the current study the oral administration of crude methanolic *F. thonningii* stem-bark did not elicit kidney damage. However, in the current study, fructose consumption did not seem to adversely affect the kidney function hence the renoprotective effects of the *Ficus thonningii* stem-bark extracts may need to be ascertained in future studies where kidney malfunction is observed.

5.8 Effect of *F. thonningii* stem-bark extracts on metabolic susbtrates

The development of features of the metabolic syndrome including impaired glucose handling, hyperglycaemia, hypertriglyceridaemia, hypercholesterolaemia and insulin resistance due to prolonged fructose feeding is well documented (Bantle, 2009). In the present study, the consumption of a high fructose diet alone or with fenofibrate or *F. thonningii* extract as interventions resulted in similar concentrations of blood glucose, plasma insulin and

cholesterol concentration as well as HOMA-IR indices in both male and female rats (Tables 4.8 and 4.9). In both male and female rats, the consumption of a high-fructose diet resulted in an increase in blood triglyceride concentration. These findings suggest that the consumption of a high fructose diet for 8 weeks induced hypertriglyceridaemia but did not elicit hyperglycaemia, hypercholesterolaemia and or insulin resistance in growing rats. In addition, fenofibrate and crude methanolic *F. thonningii* stem-bark extracts, as interventions, did not cause blood glucose and plasma cholesterol dysregulation. (Faeh et al., (2005) and Grau et al. (2018) reported that fructose consumption stimulates *de novo* hepatic lipogenesis, which results in increased blood triglyceride concentration in rodents. This corroborates with findings in the current study wherein the high-fructose diet resulted in significantly higher plasma triglyceride concentration in both male and female rats compared to that of their counterparts fed a control diet. However, the blood triglyceride concentration of rats fed the control diet were similar to that of the rats fed the high-fructose diet with either fenofibrate and or *F. thonningii* extract (both low and high dose) as interventions, suggesting that the *F. thonningii* extracts and fenofibrate attenuated a high-fructose diet-induced high plasma triglyceride concentration in both male and female rats.

Grau et al. (2018) reported that fructose-rich diets caused increased lipid storage in the liver of young rats. Findings of the current study show similarities in the liver lipid content of male rats across treatment regimens. These results suggest that a high-fructose diet or the high-fructose diet with either *F. thonningii* extracts or fenofibrate as an intervention did not alter liver lipid storage in male rats. However, in female rats, the consumption of a high-fructose diet resulted in livers with a lower lipid content compared to the liver lipid content of female rats fed a control diet suggesting that the high-fructose diet caused decreased liver lipid storage. Results from the current study are at variance with the observation by Lê et al. (2009) wherein the consumption of a diet rich in fructose was seen to increase liver lipid content. It could be speculated that the high-fat high-fructose diet reduced liver lipid content in the rats by shuttling triglycerides into circulation. Nonetheless, this typical finding requires further investigation. Interestingly, the rats fed a high-fructose diet with the low dose *F. thonningii* extract as an intervention had the highest liver lipid content compared to other treatment regimens. These results suggest that while a high-fructose diet did not induce lipid accumulation in the liver of female rats, a low dose of *F. thonningii* mediated an increase in hepatic lipid storage content which if it were to get to pathological threshold may lead to hepatic steatosis. Importantly, it can be inferred that caution must be taken when using low

doses of *F. thonningi* as a potential prophylactic agent in high-fructose diet-induced models of metabolic derangements in growing female rats as it may lead to excessive accumulation of lipids in the liver. Mechanisms through which a low dose of *F. thonningi* increases hepatic lipid storage requires further elucidation.

The following chapter highlights the main conclusion(s) drawn from the study and highlights some limitations of the study and makes some recommendations concerning future studies.

CHAPTER 6: CONCLUSION, LIMITATIONS AND RECOMMENDATIONS

6.1 Conclusion

The effects of orally administered *F. thonningii* stem-bark methanolic extract on the growth performance, glucose homeostasis, viscera morphometry, metabolic substrate content and the health of growing Sprague-Dawley (SD) rats fed a high-fructose diet were evaluated. The feeding of fructose has been demonstrated to be an effective experimental model that causes for the development of obesity and associated metabolic derangements in rodents (Bellamkonda et al., 2018). The high fructose diet alone and or with either of the interventions did not compromise the growth of the rats. However, the consumption of a high fructose diet alone for 8-weeks caused increased blood triglyceride concentration in both sexes but did not affect liver lipid content in female rats and femora density in male rats). However, consumption of the high fructose diet induced hepatic micro-steatosis and inflammation in both rat sexes. Feeding the high fructose diet did not elicit visceral adiposity, hyperglycaemia, hypercholesterolaemia, and IR in the growing SD rats. The oral administration of the low and high dose of the crude methanolic *F. thonningii* stem back extract as well as that of fenofibrate attenuated the fructose diet-induced increase in blood triglyceride concentration. Importantly, the oral administration of both the low and high dose crude methanolic *F. thonningii* stem-bark extracts protected the rats against the high-fructose diet-induced steatosis and inflammation.

The oral administration of a low dose methanolic *F. thonningii* stem-bark extract caused increased liver lipid accretion in high-fructose diet fed female rats only while the oral administration of a high dose methanolic *F. thonningii* stem-bark extracts increased the large intestine mass (absolute) and length of the rats. Crude methanolic *F. thonningii* stem-bark extracts elicited increased liver lipid content in a sexually dimorphic manner. Its use could increase the risk of the development of fatty liver disease in females. The crude methanolic *F. thonningii* stem bark extracts can potentially be used as a prophylaxis against fructose diet-mediated increased plasma triglyceride concentration, hepatic steatosis and inflammation. The protective effects of *F. thonningii* stem-bark extract against diet-induced high triglyceride concentration, steatosis and inflammation and the mechanism thereof need to be further investigated in hypertriglyceridaemic and obesity models. However, caution should be taken in the use of the low doses of crude methanolic stem-bark extracts as prophylactic agents as they may cause hepatic lipid accumulation which could lead to liver disease.

Fenofibrate reduced femora mass and density in growing male Sprague Dawley rats fed a high-fructose diet and increased liver mass (hepatomegaly) in male and female rats suggesting that fenofibrate can compromise bone and liver health. The negative effect of fenofibrate on bone and liver health necessitate further investigation using cellular and molecular techniques.

6.2 Limitations and recommendations

In the present study the consumption of a high-fructose diet apart from causing increased triglyceride concentration, hepatic steatosis and inflammation did not elicit other metabolic derangements in the rats. The failure to elicit metabolic derangements known to be caused by the consumption of a high fructose diet could have been due to the use of the "extra" calories in supporting growth since growing rats were used. In this study the mechanisms by which the methanolic *F. thonningii* stem bark extracts attenuated the high fructose diet-induced increase in plasma triglycerides, hepatic steatosis and inflammation were not interrogated. Future studies should therefore include molecular techniques such as gene expression in order to dissect the mechanism(s) behind the protective effects of the *F. thonningii* against diet-induced hypertriglyceridaemia, hepatic steatosis and inflammation.

The plasma concentration of HDL, VLDL, LDL and leptin could not be assayed due to technical limitations. Measurement of these parameters might have helped to assess the dyslipidaemic effects of dietary fructose. Analysis of these parameters should be considered in future studies. Leptin resistance and hypertension should also be assayed for in future studies as these are also said to be metabolic derangements that are known to result from the consumption of high fructose diets.

CHAPTER 7: REFERENCES

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APPENDICES

Appendix 1: Animal ethical clearance certificate

STRICTLY CONFIDENTIAL

ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2016/05/24/C

APPLICANT: Ms Y Mhosva

SCHOOL: Physiology
DEPARTMENT:
LOCATION:

PROJECT TITLE: Effect sof crude Ficus thonningii stem-bark extracts on high-cholesterol high-fructose diet fed growing Sprague Dawley (Rattus norvegicus) rats

Number and Species

40 Male 21 day old Sprague Dawley (Rattus norvegicus) and 40 Female 21 day old Sprague Dawley (Rattus norvegicus)

Approval was given for the use of animals for the project described above at an AESC meeting held on 2016/05/31. This approval remains valid until 2018/06/06.

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 is subject to any additional conditions listed below:

None

Signed: _____ (Chairperson, AESC) Date: 9 June 2016

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: 08 June 2016

cc: Supervisor: Dr E Chivandi
Director: CAS

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Appendix 2: Elisa method

Sample collection and storage

Samples should be clear and transparent and be centrifuged to remove suspended solids.

Serum: Allow samples to clot for 2 hours at room temperature or overnight at 4℃before centrifugation for 15 minutes at 1000×g. Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2 - 8℃ within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Hemolysis samples are not suitable for ELISA assay!

Cell culture supernate: Centrifuge supernate for 20 minutes to remove insoluble impurity and cell debris at 1000×g at 2 - 8°C. Collect the clear supernate and carry out the assay immediately.

Tissue homogenates: You'd better get detailed references from other literatures before assay aiming at different tissue types. For general information, hemolysis blood may affect the result, so you should mince the tissues to small pieces and rinse them in ice-cold PBS (0.01M,pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then homogenized in PBS(the volume depends on the weight of the tissue) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifugated for 5minutes at 5000×g to get the supernate.

Other biological fluids: Centrifuge samples for 20 minutes at 1000×g at 2 - 8℃. Collect the supernatant and carry out the assay immediately.

Note:

1. Samples should be used within 7 days when stored at 2-8℃, otherwise samples must be divided and stored at -20℃ (≤1month) or -80℃ (≤6months) to avoid the loss of bioactivity and contamination. Avoid repeated freeze-thaw cycles.
2. Please take the samples to room temperature (18-25℃) without extra heating before performing the assay.
3. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

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Not For Diagnostic Use

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Sample preparation

1. Elabscience is only responsible for the kit itself, but not for the samples consumed during the experiment. The user should calculate the possible amount of the samples needed in the whole test. Reserving sufficient samples in advance is recommended.
2. If the samples are not mentioned in this manual, a pre-experiment to determine the validity of the kit is necessary.
3. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected Elisa results due to the impacts of certain chemicals.
4. Due to the possibility of mismatching between antigen from other origins and antibodies used in our kits, some native or recombinant proteins from other manufacturers may not be detected by our kits.
5. Influenced by factors including cell viability, cell number or sampling time, molecular from cells culture supernatant may not be detected by the kit.
6. Grossly hemolyzed samples are not suitable for use in the assay.
7. Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

Other supplies required

Microplate reader with 450nm wavelength filter
High-precision transferpetor, EP tubes and disposable pipette tips
37℃ Incubator
Deionized or distilled water
Absorbent paper
Loading slot for Wash Buffer

Reagent preparation

Bring all reagents to room temperature(18-25℃) before use.

Wash Buffer - Dilute 30 mL of Concentrated Wash Buffer into 750 mL of Wash Buffer with deionized or distilled water. Put unused solution back at 4℃. If crystals have formed in the concentrate, you can warm it with 40℃water bath (Heating temperature should not exceed 50℃) and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.

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