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THE EFFECT OF GARLIC (*ALLIUM SATIVUM*) POWDER ON GROWING SPRAGUE DAWLEY RATS FED A HIGH-FAT HIGH-FRUCTOSE DIET

Tshepiso Ngoetsana

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

Johannesburg, 2019

DECLARATION

I, Tshepiso Ngoetsana, declare that the entire dissertation is my work, with assistance by the acknowledged persons. It is being submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa, in fulfilment of the requirements for the degree of Master of Science in Medicine. It has not been submitted before for any examination or degree at this or 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).

……

Tshepiso Ngoetsana

Signed on the……..day of……2019

DEDICATION

To my loving and supportive family

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

ABSTRACT

Obesogenic [high- fat high- fructose (HFHF)] diets increase the prevalence of obesity and associated metabolic derangements (MD) in children. Conventional medicaments used to manage obesity besides being costly elicit side effects hence communities rely on ethnomedicines. Garlic, an ethnomedicine, has antioxidant, hypoglycaemic and hypolipidaemic properties. This study evaluated the prophylactic potential of garlic powder (GP) in growing Sprague-Dawley (SD) rats against diet-induced MD. Eighty 21-day old SD rats were randomly allocated to and administered treatment regimens: group 1- rat chow (SRC) + drinking water + gelatine cube (PGC); group 2- SRC with 2% beef tallow and 20% fructose solution (HFHF) + PGC; group 3- HFHF + fenofibrate (100mg/kg bwt/day) and group 4- HFHF + GP (100mg/kg bwt/day) for 8 weeks. Growth, glucose tolerance, metabolic substrate content, viscera morphometry, and general health profile were determined. The HFHF diet, fenofibrate, and GP did not affect ($P > 0.05$) growth performance. The haematocrit of males was decreased ($P< 0.05$) by the HFHF diet. The HFHF diet did not affect ($P > 0.05$) the area under the curve (AUC) of the oral glucose tolerance test. Fenofibrate and GP did not affect ($P > 0.05$) the AUC of female rats but increased the AUC ($P < 0.05$) of male rats. In both sexes, treatment regimens had no effect ($P > 0.05$) on plasma insulin and cholesterol concentration and HOMA-IR. In females, fenofibrate increased ($P < 0.05$) blood glucose concentration compared to that of counterparts fed the HFHF diet. The HFHF diet increased ($P < 0.05$) plasma triglyceride concentration in both sexes but did not affect ($P > 0.05$) liver lipid content of females, however it decreased ($P < 0.05$) that of males. The HFHF diet-induced hypertriglyceridaemia was prevented by fenofibrate in males. Garlic powder and fenofibrate increased ($P < 0.05$) the liver lipid content in both sexes. In females, the HFHF diet caused hepatic steatosis and inflammation ($P < 0.05$). Garlic powder and fenofibrate protected against the HFHF diet-induced hepatic steatosis and inflammation. Fenofibrate increased ($P < 0.05$) liver mass in both sexes but increased ($P < 0.05$) kidney mass of males. The HFHF diet increased ($P < 0.05$) visceral and epididymal adiposity in the rats. Garlic powder and fenofibrate protected the females against the visceral adiposity. The diet-induced epididymal adiposity was prevented by garlic powder. The HFHF diet decreased BUN ($P < 0.05$) and fenofibrate increased ($P < 0.05$) the BUN: creatinine ratio.

The HFHF showed sexual dimorphism in eliciting metabolic derangements. In both sexes, GP protected against the HFHF diet-induced metabolic derangements in a sexually dimorphic manner. In growing children, garlic powder and fenofibrate can potentially be used to protect against some components of the HFHF diet-induced MD but should be used with caution as they might cause adverse health outcomes.

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"Coming together is a beginning. Keeping together is progress. Working together is a success" (Henry Ford) – this research is a result of the passion that was brought forward for its success through teamwork. I would like to extend my gratitude to the following individuals and groups for their assistance.

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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
FFAs:	Free fatty acids
FGC:	Fenofibrate in gelatine cube
FDW:	Fructose solution
GGC:	Garlic in gelatine cube
GIT:	Gastrointestinal tract
GP	Garlic powder
HDL-C:	High-density lipoprotein cholesterol
HFHF:	High-fat High-fructose diet
HOMA-IR:	Homeostatic model of insulin resistance
IDF:	International Diabetes Federation
LDL-C:	Low-density lipoprotein cholesterol
LI:	Large intestine
MD:	Metabolic derangement
MetS:	Metabolic syndrome
OGTT:	Oral glucose tolerance test
PGC:	Plain gelatine cube
PDW:	Plain drinking water
PPAR-α:	Peroxisome proliferators activated receptor alpha
SI:	Small intestine
ß:	Beta
%TBM:	Percent of terminal body mass
rTL:	Relative to tibia length
VLDL:	Very low density lipoprotein
w/v:	weight/volume

WHO:	World Health Organization

CHAPTER 1: INTRODUCTION AND JUSTIFICATION

1.0 Preview of the dissertation

This dissertation comprises of seven chapters, namely the introduction, literature review, materials and methods, results, discussion, conclusion, recommendations and limitations, and references.

Chapter one: introduces the problem of obesity in growing children and its (obesity) as one of the major drivers of metabolic derangements such as metabolic syndrome (Mets) in children. The chapter highlights the challenges associated with the use of conventional pharmacological agents in the management of diet-induced obesity and the attendant metabolic diseases, then suggests and justifies the need to evaluate the potential of ethnomedicines to ameliorate diet-induced metabolic derangements using growing rat models to mimic the problem of obesity in growing children. This chapter concludes by stating the aim, specific objectives and the study hypothesis.

Chapter two: this chapter gives a detailed review of the literature pertinent to the study. Specifically, it gives global, regional and local statistics with regard to the problem of obesity and metabolic derangement such as metabolic syndrome in children and adolescents. It brings to the fore the issue of poor dietary habits (consumption of energy-dense food - with saturated fats and sweeteners such as fructose) as one of the major causes of obesity and its associated metabolic diseases in growing children. The shortfalls on the models that have been used to study metabolic derangements are discussed. New models and the use of plant-derived ethnomedicines are described with emphasis placed on the need to interrogate the prophylactic effects of these ethnomedicines against diet-induced metabolic derangements as well as the need to establish their safety. The chapter then discusses garlic as one of the ethnomedicines with the potential to prevent the diet-induced metabolic derangements by bringing to the fore the health beneficial biological activities (hypolipidaemic, hypoglycaemic and antioxidant) of garlic powder.

Chapter three: this chapter describes in detail the materials used in executing the study as well as the methods used for the various analyses and assays on the samples and tissues collected.

The chapter also details how data – both parametric and non-parametric was analyzed.

Chapter four: the chapter is a presentation of the results obtained from the study. The results presented in graphic and tabular form are also described with the effects of the high-fat high-fructose diet as well as the fenofibrate (positive control) and garlic powder well narrated in a manner that allows the reader to get an understanding of the key findings.

Chapter five: this chapter largely dwells on giving meaning to the study findings. It focuses on giving meaning to the results, their possible application viz the study's aim and objectives and also compares the findings to other relevant studies. The chapter attempts to explain the possible mechanisms of how garlic powder and fenofibrate exerted beneficial effects and/or failed to exert such effects.

Chapter six: this chapter states the major conclusion(s) drawn from the study, highlights some areas of weakness and makes some recommendations regarding future studies.

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

1.1 Introduction

Obesity has become a global epidemic among children and adolescents (Pienaar and Kruger, 2014). Its prevalence in children and adolescents in developing countries has increased (Weiss *et al.*, 2004; Mandal and Mandal, 2012) from 8.1% to 12.9% for boys and 8.4% to 13.4% for girls between the years 1980 and 2013 (Ng *et al.*, 2014), while in South Africa it increased from 12.5% to 16.7% (Pienaar, 2015). Obesity is the excessive accumulation of fat in the body. There are many factors that contribute to the development of obesity in growing children such as genetic, epigenetic and compromised maternal nutrition during pregnancy (Mollentze, 2006). In growing children, one of the major drivers of obesity is the consumption of diets rich in saturated fats and laden with artificial sweeteners such as fructose, as well as sedentary lifestyles (Sidik and Ahmad, 2004; Mollentze, 2006). Sedentary lifestyles and poor dietary habits typified by the consumption of diets rich in saturated fats and fructose has been and continues to increase due to urbanization (Mchiza and Maunder, 2013). The consumption of such obesogenic diets results in an increase in plasma triglyceride, total cholesterol, and low-density lipoprotein cholesterol (LDL-C) concentration (Sidik and Ahmad, 2004). Importantly, such high-calorie diets result in abnormal fat accumulation in the liver that can cause hepatic steatosis (Gonzalez *et al.*, 2013). Besides negatively affecting the liver, these obesogenic diets are a major cause of other metabolic derangements, for example, impaired glucose regulation, hypertension (Vanhala *et al.*, 1998; Sidik and Ahmad, 2004) and metabolic syndrome (Zhou *et al.*, 2014). Han and Lean (2015) contend that metabolic syndrome is a condition characterized by the co-existence of several major risk factors for cardiovascular disease, hyperglycemia, and dyslipidemia. The dyslipidemia is characterized by either reduced high-density lipoprotein cholesterol and or raised triglycerides (Han and Lean, 2015). Metabolic syndrome has a reported global prevalence ranging from 10 to 50% (Khanam *et al.*, 2011). It (Mets) results from an accumulation of metabolic derangements (MDs) and is typified by the presence of insulin resistance, hyperglycemia, atherogenic dyslipidemia, and hypertension (Eckel *et al.*, 2005; Dissard *et al.*, 2013). The increased occurrence of these diet-induced MDs has been associated with an increase in the prevalence of MetS in children and adolescents (Juárez-López *et al.*, 2010). The high fructose diet-induced insulin resistance increases the risk of ectopic deposition of lipids and tissue-specific lipotoxicity (Le *et al.*, 2009). In humans, the dyslipidaemia associated with the

consumption of a high fructose diet stems from increased hepatic *de novo* lipogenesis and decreased clearance of very-low-density lipoprotein cholesterol (Faeh *et al.*, 2005).

The increase in the prevalence of diet-induced obesity among children and adolescents and its association with the increased risk for MDs and MetS is a major cause of concern globally. Many conventional pharmacological agents are used to manage obesity, various MDs and MetS, for example, fenofibrate and metformin, which are used to manage high blood lipids and high blood glucose concentration, respectively (Jeong and Yoon, 2009; Lipska *et al.*, 2011). However, these conventional pharmacological agents are relatively inaccessible for some communities, are expensive and elicit deleterious side effects (Erasto *et al.*, 2005; Aksay *et al.*, 2007). Importantly, each of these conventional pharmacological agents have a specific target thus they fail to adequately assist patients suffering from MDs and MetS since metabolic disorders result from a multiplicity of mechanisms (Kraja *et al.*, 2010; Vella *et al.*, 2010) and would be relieved by interventions with multiple health beneficial biological activities. As a result of the shortcomings associated with the use of conventional pharmacological agents to manage diet-induced MDs and disease in adults, children, and adolescents, communities (globally) rely on plant-derived ethnomedicines to manage these metabolic derangements and associated diseases (Benzie and Wachtel-Galor, 2011). These plant-derived ethnomedicines are natural and are deemed safe (Erasto *et al.*, 2005; Lembede *et al.*, 2018). In addition to their multifactorial benefits, the search and development of ethnomedicines is critical to the development of indigenous knowledge systems (Mander, 1998; Moyo *et al.*, 2015). Many plants are exploited as ethnomedicines and garlic (*Allium sativum*), a common spice, is one of the plants exploited for its ethnomedicinal value (Hosseini and Hosseinzadeh, 2015).

Garlic (*Allium sativum*) which belongs to the genus *Allium* contains potent bioactive phytochemicals inclusive of saponins, alkaloids, tannins, glycosides and flavonoids (Ameh *et al.*, 2013; Garba *et al.*, 2013) with health beneficial activities. The health beneficial biological activities of these phytochemicals include properties such as being hypoglycemic, hypocholesterolemic, antiobesity (Lee *et al.*, 2011), antioxidant and anti-cancer (Joo *et al.*, 2013). Garlic's health beneficial effects are believed to be due to the presence, in its extracts, of alliin, allicin, ajoene, diallyl disulfide, diallyl trisulfide, allyl methanethiosulfonate, and S-allyl cysteine, which are organosulphur phytochemicals (Yun *et al.*, 2014).

An array of research evidence has shown raw garlic to be effective at reducing hyperglycemia and to attenuate diabetes mellitus-induced dyslipidemia and oxidative stress in both humans and animal models (Liu *et al.*, 2005; Thomson *et al.*, 2006; Drobiova *et al.*, 2011). Research has demonstrated garlic to be effective at reducing the mass of white adipose tissue and body mass (Lee *et al.*, 2011; Joo *et al.*, 2013). In mice, fresh garlic has been shown to lower serum lipids by reducing lipid absorption from the gastrointestinal tract and also diminish hepatic LDL-cholesterol (Djankpa *et al.*, 2012). Additionally, the health beneficial biological activities, its demonstrated efficacy at reducing white adipose tissue and body mass, as well as its ability to lower lipid absorption and hepatic LDL-cholesterol synthesis, are potentially useful for prophylactic and therapeutic purposes (Ameh *et al.*, 2013).

1.2 Justification of the study

Studies that interrogated the potential beneficial effects of garlic in diet-induced metabolic derangements typically made use of adult animal models (Elkayam *et al.*, 2003; Thomson *et al.*, 2016). However, the prevalence of obesity, metabolic derangements, and diseases due to poor dietary practices are increasing in growing children and adolescents (Mandal and Mandal, 2012) thus such adult animal models do not give insight into the potential of garlic in growing children. Importantly, in studying the potential of garlic to mitigate metabolic derangements, pharmacological agents, for example, streptozotocin and alloxan, have been used to chemically induce diabetes mellitus and obesity, respectively (El-Demerdash *et al.*, 2005; Eidi *et al.*, 2006; Bokaeian *et al.*, 2010).

These chemically induced metabolic models in rats do not mimic the real-life situation where metabolic derangements and disease are diet-induced in children; especially the consumption of obesogenic diets (Panchal and Brown, 2011). Other studies have used different strains of animals testing for the prophylactic efficacy of garlic other than Sprague Dawley rats and the majority of these studies utilized male animals than both sexes (Elkayam *et al.*, 2003; Thomson *et al.*, 2016). In evaluating the potential prophylactic efficacy of garlic against obesogenic diet-induced metabolic derangements, there is a need, therefore, to make use of both male and female growing rats as sex differences are known to cause differences in response to prophylaxis (Klein, 2013). Research has amply demonstrated that women turn to have a high-fat mass compared to men and

that the high fructose diet-mediated hypertriglyceridemia is dampened in human females suggesting that their sex hormones may be exerting a protective effect (Couchepin *et al.*, 2008; Stanhope *et al.*, 2008). Thus feeding both male and female rats with a high fructose diet and garlic powder is most likely to provide a platform to observe possible sexual dimorphism in regard of the high-fructose high-fat diet and garlic as an intervention. Most of the research on the potential of ethnomedicines has focused on finding curative ethnomedicines with little if any of their potential prophylactic properties. This focus is true with regards to garlic: preceding research has focused on its therapeutic potential but not on its potential protective effect against obesogenic (high fat; high fructose) diet-induced MetS (Simmons *et al.*, 2010; Masjedi *et al.*, 2013) in growing rats modelling growing children that are fed such obesogenic diets. Therefore, the prophylactic potential of garlic powder needs to be interrogated more particularly in growing rats subjected to poor dietary habits mimicking growing children fed high-fat high-fructose diets (Joo *et al.*, 2013; Thomson *et al.*, 2016).

1.3 Aim and objectives of the study

This study evaluated the prophylactic potential of orally administered garlic powder against the development of metabolic derangements in growing Sprague Dawley rats fed a high-fat high fructose diet.

The objectives of the study were to:

a. quantitatively determine total phenols in garlic powder used in the study.

b. determine the effects of orally administered garlic powder in growing Sprague Dawley rats fed a high-fat high-fructose diet on:

i. growth performance by evaluating body mass gain and linear growth.

ii. packed cell volume and erythrocyte osmotic fragility.

iii. the ability of the rats to tolerate an oral glucose challenge.

iv. blood cholesterol, glucose, and triglyceride concentration and hepatic lipid content.

v. plasma insulin concentration and HOMA-IR.

vi. gastrointestinal tract [(GIT) stomach, small and large intestine] and other viscera (liver, kidneys, pancreas, epididymal fat, visceral fat) macro-morphometry as well as liver and kidney micro-morphometry.

vii. surrogate markers of the liver function [alkaline phosphatase (ALKP) and alanine aminotransferase (ALT)] and of kidney function (serum creatinine and urea concentration).

1.4 Hypothesis

H_0: Garlic powder has no effect on the growth performance, tolerance to an oral glucose load, blood, and liver metabolic substrate contents, plasma insulin concentration, viscera morphometry and the surrogate markers of liver and kidney function of growing Sprague Dawley rats fed a high-fat high-fructose diet.

H_1: Garlic powder affects the growth performance, tolerance to an oral glucose load, blood, and liver metabolic substrate contents, plasma insulin concentration, viscera morphometry and the surrogate markers of liver and kidney function of growing Sprague Dawley rats fed a high-fat high-fructose diet.

The next chapter gives a detailed review of the literature pertinent to the study. The chapter highlights the issue of poor dietary habits as one of the major causes of obesity and its associated metabolic diseases.

CHAPTER 2: LITERATURE REVIEW

2.0 Introduction

Globally, the prevalence of obesity is increasing in children and adolescents (World Health Organization, 2011). Obesity is associated with the onset of metabolic syndrome that increases the risk of developing type II diabetes mellitus and cardiovascular diseases (Despres and Lemieux, 2006). Poor dietary habits (consumption of a diet high in fat and fructose) and sedentary lifestyles are some of the major causes of obesity (Sidik and Ahmad, 2004; Mchiza and Maunder, 2013). Interventions that can be used to manage obesity and its associated complications are lifestyle changes (increased physical activity and healthy dietary habits), conventional pharmacological agents and plant-derived ethnomedicines.

2.1 Obesity

Obesity is a condition in which excess fat accumulates in the body to a point where adverse health effects manifest (World Health Organization, 2000). Polygenetic predisposition and environmental influences, particularly poor dietary habits and sedentary lifestyles are known to be among the major drivers of the increased prevalence of obesity (Jurgens *et al.*, 2005). With regards to poor dietary habits, the consumption of high-fat high-fructose diets has been proven to be one of the main causes of obesity (Jurgens *et al.*, 2005; Panchal *et al.*, 2011). According to the World Health Organization (World Health Organization, 2011), the consumption of high-fat high-fructose diet is associated with increased adiposity. Compared to glucose, fructose does not stimulate insulin secretion from the endocrine pancreas which (failure to stimulate insulin secretion) results in subnormal blood leptin concentration (Tappy and Lê, 2010). Leptin is required for regulating energy balance through the inhibition of hunger, thus lower than normal leptin concentration results in body mass gain and increased adiposity (Sáinz *et al.*, 2015). An increase in adiposity has been shown to increase the risk of the development of metabolic derangements and diseases (Sáinz *et al.*, 2015). While the WHO has methods for use to screen for obesity; some of the methods are beset with inaccuracies, for example, body mass index fails to distinguish between muscle and fat mass and also does not represent fat distribution across the body (Nutrition, 2003; Kelishadi, 2007). Imaging techniques such as magnetic resonance imaging and computer-assisted tomography can be used to accurately diagnose obesity but these techniques are expensive and are largely inaccessible to most communities (Lee *et al.*, 2004).

Visceral adiposity is one of the major risk factors for the development of insulin resistance (Juárez-López *et al.*, 2010). Insulin resistance mediated by obesity increases risk and susceptibility to metabolic diseases such as type II diabetes mellitus, hypertension, atherosclerosis (Grundy, 1998), atherogenic dyslipidemia, coronary heart disease and hyperuricemia (Flores-Huerta *et al.*, 2009; Juárez-López *et al.*, 2010). The metabolic derangements and diseases constitute a cocktail of components that make upon MetS (Juárez-López *et al.*, 2010).

2.2 Metabolic syndrome

Metabolic syndrome (MetS) is a cluster of various metabolic derangements and it's a risk factor for the development of type II diabetes mellitus, venous thromboembolism and cardiovascular diseases (Juárez-López *et al.*, 2010; Simmons *et al.*, 2010). It (MetS) is characterized by the presence of several metabolic derangements inclusive of central adiposity, insulin resistance, hyperglycemia, impaired glucose tolerance, dyslipidemia, non-alcoholic fatty liver disease (NAFLD) and hypertension (Amato *et al.*, 2017). Complications resulting from MetS constitute the leading non-communicable diseases that contribute to childhood and adulthood mortality (World Health Organization, 2014). The global prevalence of MetS is reported to be 25% (Mendis *et al.*, 2015). The diagnostic criteria for MetS vary depending on the authority quoted. The WHO states that in human adults the presence of insulin resistance and any two of the following an increased waist-to-hip ratio, hypertriglyceridemia and or low HDL-cholesterol concentration constitutes MetS (Onis *et al.*, 2007). However, the National Cholesterol Education Program's Adult Treatment Panel III (ATP III) criteria for MetS diagnosis includes the presence of any three of the following risk factors increased waist circumference, high blood TGs concentration, lower HDL-cholesterol concentration and or high fasting blood glucose concentration (Eckel *et al.*, 2005).

While according to Rashidi *et al.* (2014), the criterion for diagnosing MetS in children are not well defined, Cruz *et al.* (2004) and argues that paediatric MetS exists when three of the following are present: abdominal obesity with a waist circumference of $\geq 90^{th}$ percentile, low HDL-C level of $\leq$ 40mg/dl, hypertriglyceridemia of $> 90^{th}$ percentile and/or impaired glucose

tolerance. Lambert *et al.* (2004) recommend the consideration of fasting insulin as a component of MetS by using percentiles. The difference in the criteria used to diagnose pediatric MetS makes it difficult to accurately diagnose it in children. This challenge is further worsened by the difference in growth patterns, variation in sexual maturation and development as well as ethnicity (Ford, 2005).

2.2.1 Central obesity

Central adiposity is an upper-body obesity (Nyamdorj *et al.*, 2009) which increases the risk of developing type II diabetes mellitus and atherogenic dyslipidemia (Eckel *et al.*, 2005). A waist circumference of ≥102cm in males and ≥88cm in females denote intra-abdominal adiposity which equates to obesity (Grundy *et al.*, 2004). Compared to the BMI which only detects whether a person is underweight, has normal weight, overweight and or obese, the use of waist circumference points to the location of the excess fat which is more useful in terms of health prognosis (World Health Organization, 2011). In addition to the use of the waist circumference and BMI, waist-to-hip ratio is one of the measures of adiposity. A waist-to-hip ratio of >0.90 (males) and >0.85 (females) is diagnostic for central obesity (Grundy *et al.*, 2004).

Adipose tissue is an endocrine organ that stores energy in the form of triacylglycerols and secretes adipokines (Flores-Huerta *et al.*, 2009). These adipokines are pro- and anti-inflammatory cell signalling molecules (Flores-Huerta *et al.*, 2009). Pro-inflammatory adipokines promote inflammation and modulate insulin resistance while the anti-inflammatory adipokines mitigate inflammation (Dinarello, 2000). Importantly, pro-inflammatory adipokines interfere with insulin signaling in peripheral tissues leading to insulin resistance (Esser *et al.*, 2014). It thus can be argued that increased central adiposity that results from increased adipose tissue which secretes signalling molecules such as pro-inflammatory adipokines increase the risk of developing insulin resistance and MetS (Phillips and Perry, 2013). The increased central adiposity causes increased lipolysis which translates to over-supply of FFAs to the liver (Eckel *et al.*, 2005). An abnormal oversupply of FFAs to the liver impairs key metabolic processes resulting in hyperinsulinemia, glucose intolerance and hypertriglyceridemia (Eckel *et al.*, 2005).

2.2.2 Insulin resistance and hyperglycemia

Insulin is known to stimulate glucose uptake by adipose tissue and skeletal muscle and to stimulate hepatic glycogen storage as well as to inhibit hepatic glycogenolysis and gluconeogenesis (Pessin and Kwon, 2013). It promotes lipogenesis in adipose tissue and hepatocytes but diminishes lipolysis of adipose tissue (Pessin and Kwon, 2013). Insulin resistance is a condition wherein the ability of insulin to activate insulin receptors in key metabolic tissues becomes inadequate (Eckel *et al.*, 2005). This insulin resistance mediated inadequacy manifest in the form of poor glucose uptake by cells and hyperglycemia (Eckel *et al.*, 2005). A model used to assess the degree of insulin resistance is termed the homeostasis model assessment of insulin resistance (HOMA-IR). A HOMA-IR of >4.65 is indicative of insulin resistance (Sala-Vila *et al.*, 2011). The sustained and rapid secretion of insulin resulting from the consumption of large quantities of carbohydrates as well as the chronic circulation of excessive free fatty acids are known causes of insulin resistance (Lee *et al.*, 1994; Eckel *et al.*, 2005). The excess consumption of a high-caloric diet initiates adipocyte hypertrophy and hyperplasia that results in cellular stress which in turn causes oxidative stress and inflammatory responses in adipose tissue (Pessin and Kwon, 2013). These inflammatory responses become self-generating which leads to elevated local and systemic levels of different proinflammatory cytokines inclusive of tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-1β and CC-chemokine ligand 2 (CCL2) eventually, that cause insulin resistance (Pessin and Kwon, 2013). Patients with diabetes mellitus and or insulin resistance tend to depend more on adipose tissue as a source of energy (Galic *et al.*, 2010). This over-dependence on fat results in excessive lipolysis which effectively result in dyslipidemia characterized by increased plasma triglyceride and low HDL-cholesterol concentration (Cruz *et al.*, 2004).

2.2.3 Dyslipidaemia

Diet-induced hyperlipidemia has been demonstrated to stimulate the sterol regulatory element-binding protein-1 (SREBP-1c) (Kim *et al.*, 2004). The SREBP-1c is a critical transcription factor which regulates genes that are necessary for the *de novo* lipogenesis by activating lipogenic enzymes that catalyze triglyceride and cholesterol synthesis (Kim *et al.*, 2004; García-Ruiz *et al.*, 2013). Abnormally high plasma concentrations of LDL-cholesterol and VLDL-cholesterol

remnants, hypertriglyceridemia and low levels of HDL-cholesterol (Tangvarasittichai, 2015) are the major hallmarks of dyslipidemia in individuals presenting with MetS. Low-density lipoprotein-cholesterol and VLDL-cholesterol are atherogenic and predispose individuals to cardiovascular complications such as coronary artery disease and hypertension (Tangvarasittichai, 2015). The plasma concentration of these LDL-cholesterol and VLDL-cholesterol has been shown to increase with high consumption of diets rich in fructose and animal fat (Tangvarasittichai, 2015). While hepatic dyslipidemia is characterized by the accumulation of fat in the liver (Lemke *et al.*, 2008). A plasma triglyceride concentration level of ≥1.7mmol/L and or HDL-cholesterol of <0.9mmol/L in males and <1.0mmol/L in females is diagnostic of dyslipidemia (Eckel *et al.*, 2005).

2.2.4 Non-alcoholic fatty liver disease

Globally, an estimated 25% of the adult population has non-alcoholic fatty liver disease [NAFLD; (Younossi *et al.*, 2017)]. Paediatric NAFLD is reported to range from 3-12% (Bush *et al.*, 2017). Non-alcoholic fatty liver disease is a metabolic disorder that is characterised by the excessive deposition of fat in the liver parenchyma, that is not related to excessive alcohol intake, an autoimmune process, medication, or infection (de Moura *et al.*, 2008; Bush *et al.*, 2017). Although the pathogenesis of NAFLD is poorly understood, it is well documented that its development is as a result of the sequence variation and or alteration in the patatin-like phospholipase domain-containing protein 3 (PNPLA3) with dietary interventions (Huang *et al.*, 2010). This PNPLA3 causes liver injury and the deposition of triglycerides in the liver (Huang *et al.*, 2010). It (NAFLD) is regarded as a hepatic onset of MetS (Amato *et al.*, 2017). It is characterized by hepatic steatosis of ≥5% in the absence of other etiologies of liver disease (Younossi *et al.*, 2016). The disease (NAFLD) can be diagnosed by histological analysis of the liver tissue (Younossi *et al.*, 2016). Non-alcoholic fatty liver disease is the leading cause of chronic liver disease in children and adolescents (Burgert *et al.*, 2006) and it can progress to non-alcoholic steatohepatitis [(NASH); (Lozano *et al.*, 2016)]. Non-alcoholic steatohepatitis manifests with inflammation and ballooning of hepatocytes and elevated plasma activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Burgert *et al.*, 2006).

2.2.5 Diabetes mellitus

Diabetes mellitus is a metabolic disease that is characterized by the body's inability to regulate blood glucose concentration and is ranked the third most common non-communicable chronic disease (Pettitt *et al.*, 2014). In 2010 the prevalence of diabetes mellitus was 6.4% among adults globally and it was expected to rise to 7.7% by 2030 (Shaw *et al.*, 2010). In South Africa, 4.5% of the adult population had type II diabetes mellitus in 2010 (Shaw *et al.*, 2010). The prevalence of diabetes mellitus in South African adults is expected to increase to 4.9% by 2030 (Shaw *et al.*, 2010). There are two types of the disease: type 1 diabetes mellitus which results from autoimmune destruction of the endocrine pancreatic beta cells (World Health Organization, 1999; Herold *et al.*, 2002) and type II diabetes mellitus that results from insulin resistance (Neergheen-Bhujun *et al.*, 2013). Both types of diseases are typified by an increased blood glucose concentration that manifests as hyperglycemia (World Health Organization, 1999). Obesity, particularly characterized by increased central adiposity increases the risk of developing type II diabetes mellitus (Bakker *et al.*, 2000). Research has made use of several models to study diabetes mellitus and other metabolic diseases.

2.3 Models of metabolic derangements

Due to the multifactorial nature of metabolic diseases, various models have been used to evaluate potential therapeutic agents using animal models (Russell and Proctor, 2006; Tangvarasittichai, 2015). Evidence exists in the literature that most of these studies largely made use of adult male animal models. Some of the major models developed for interrogating various aspects of metabolic diseases include chemical, genetic and diet-induced rat models.

2.3.1 Chemically-induced models

Streptozotocin is an alkylating antineoplastic agent which when administered in low doses is used to treat some pancreatic tumours (Bolzán and Bianchi, 2002) but is toxic to endocrine pancreatic beta cells that produced insulin. In medical research, it has been used and continues to be used to produce an animal model for hyperglycaemia when administered in a large dose (Shiomi *et al.*, 2003; Liu *et al.*, 2005) and or diabetes mellitus with multiple low doses (Bokaeian

et al., 2010). By virtue of it being a glucose analogue, streptozotocin utilizes GLUT-2 transporters to enter the pancreatic beta cells (Panchal and Brown, 2011). Once inside the pancreatic β-cells, it damages the cells rendering them defective thus compromising the synthesis and secretion of insulin (Panchal and Brown, 2011). The damage to pancreatic β-cells then manifest as hyperglycemia and decreased tolerance to a glucose challenge (Eckel *et al.*, 2005). As a result of its effects in the pancreas, streptozotocin, therefore, can and is being used in research experiments where potential therapeutic agents can be tested (Wu and Yan, 2015). In other chemically-induced diabetic rat models, a single dose of alloxan injection induces selective necrosis to the pancreatic β-cells (Panchal and Brown, 2011). Thus, similar to streptozotocin, alloxan is used in the induction of type I diabetes (Mahesar *et al.*, 2010). However, the major criticisms to the use of these chemically-induced diabetic models are that they are only suitable for investigating type I diabetes mellitus and not the type II which (type II diabetes mellitus) is one of the main complication in diet-induced metabolic derangements particularly MetS (Panchal and Brown, 2011).

2.3.2 Genetic models

Majority of the genetic models of the constituents of MetS are based on monogenic mutations (Bertram and Hanson, 2001) such as the Otsuka Long-Evans Tokushima fatty rats, the C57BL/6J-ob/ob mice and Goto-Kakizaki rats (Panchal and Brown, 2011). The monogenic-driven mutation models are best suited for assessing the genetically-mediated mechanisms involved in the development of metabolic dysfunctions (Panchal and Brown, 2011; Lehnen *et al.*, 2013). However, these models fail to mimic the multi-factorial nature of metabolic dysfunctions (Buettner *et al.*, 2006; Lehnen *et al.*, 2013). In addition to these genetic models, nutritional models have also been established (Shirouchi *et al.*, 2007).

2.3.3 Diet-induced models

Worldwide nutrition has received attention not just for its critical contribution to normal growth and development but for its role in contributing to the pathogenesis of obesity which is a risk

factor for the development of metabolic diseases inclusive of MetS. High-calorie rich diets, for example, high-fat and high-fructose diets, are strongly associated with body weight gain and increased visceral adiposity and derangement of the plasma lipid profile (Huang *et al.*, 2004; Dupas *et al.*, 2016). It has since been established that the chronic consumption of diets rich in fat and fructose is a risk factor for the development of obesity (Astrup, 2001). Urbanization and the adoption of a western lifestyle have resulted in a shift from more natural and less processed food to the consumption of high-fat (saturated) and high-fructose diets (Mchiza and Maunder, 2013). Diets rich in saturated fat and fructose have been shown to induce insulin insensitivity in tissues (Panchal *et al.*, 2011) and to cause increased hepatic triglyceride accretion (Schindler and Felber, 1986; Lozano *et al.*, 2016). Importantly, the consumption of diets rich in saturated fats and fructose has been shown to result in obesity (Padwal, 2014) that increases the risk of developing metabolic diseases (Sidik and Ahmad, 2004).

Fructose is a naturally occurring monosaccharide sugar found in fruits and honey (Tappy and Lê, 2010). It can be produced industrially by hydrolizing corn starch (Parker *et al.*, 2010). The industrially produced fructose is widely used as a food and beverage additive and its excessive consumption in such food is known to alter plasma lipid profile causing hypertriglyceridemia in humans (Tappy and Lê, 2010) while in rodents it has been reported to lead to the development of obesity, diabetes mellitus and dyslipidemia (Tappy and Lê, 2010). The absorption of fructose into the jejunum enterocytes is facilitated by GLUT-5, a fructose transporter. Unlike that of glucose, fructose absorption is not dependent on the hydrolysis of ATP (Tappy and Lê, 2010). Following its absorption in the liver fructose-1-phosphatase converts fructose to glyceraldehyde-3-phosphate and dihydroxyacetone phosphate (Rutledge and Adeli, 2007). The two 3-carbon substrates are then used in the *de novo* synthesis of triglycerides (Rutledge and Adeli, 2007). Compared to that of fructose, the metabolism of glucose is catalyzed by phosphofructokinase making the *de novo* triglyceride synthesis less favored due to the rate-limiting effects of phosphofructokinase (Rutledge and Adeli, 2007). The practical implication is that the consumption of high-fructose diets favours faster *de novo* lipid synthesis which results in the development of increased adiposity, dyslipidaemia and related metabolic diseases (Tappy and Lê, 2010). Due to their metabolic effects, high-fructose as well as high-fat diet models have been and continue to be used as models that best mimic diet-induced metabolic diseases in growing children and adolescents.

Many studies that have relied on diet-induced models largely made use of adult animals to evaluate the efficacy of pharmacological agents in the treatment of diet-induced metabolic derangements. Some of the therapeutic pharmacological agents used in such studies, as positive controls, include statins which lower the blood lipid content (Hebert *et al.*, 1997), metformin, a blood glucose concentration lowering agent (Vella *et al.*, 2010) and fenofibrate which reduces plasma lipid concentration (Kraja *et al.*, 2010).

2.4 Fenofibrate

Due to its established hypolipidaemic effects (Yoon *et al.*, 2003), fenofibrate is one of the conventional pharmacological agents widely used to improve plasma lipid profile in both obesity and MetS (Kraja *et al.*, 2010). According to Jeong *et al.* (2004) fenofibrate exerts its health beneficial effects by activating the peroxisome proliferator-activated receptor alpha (PPAR-α). The latter (PPAR-α) regulates the expression of various genes that encode enzymes such as lipoprotein lipase, acetyl-Co-A synthetase, and oxidase which catalyze reactions pertinent to lipid metabolism (Valasek *et al.*, 2007; Kraja *et al.*, 2010). Lipoprotein lipase catalyzes the hydrolysis of plasma triglycerides (Kersten, 2014) while acetyl-Co-A synthetase promotes fatty acid synthesis and uptake by tissues (Ward, 2015). Acyl-Co-A oxidase catalyzes the oxidation of fatty acids (Ward, 2015). Additionally, fenofibrate mediates the prevention of cholesterol absorption from the small intestinal lumen into the lymphatic system hence its hypocholesterolemic effects (Valasek *et al.*, 2007). It also increases the formation of HDL, which is used to transport cholesterol from the blood to the liver, effectively lowering the concentration of circulating cholesterol (Chapman, 2006; Valasek *et al.*, 2007).

The use of conventional pharmacological agents in the management of metabolic derangements and diseases is beset with drawbacks particularly due to their targeting specific disorders, for example, metformin reduces blood glucose concentration (Vella *et al.*, 2010) while fenofibrate reduces plasma lipid concentration (Kraja *et al.*, 2010) instead of combating the several mechanisms that characterize the nature and pathogenesis of metabolic diseases such as diabetes mellitus and MetS. Plant-derived ethnomedicines due to the multiplicity of health beneficial biological and pharmacological activities (Joo *et al.*, 2013) from their phytochemicals could be used to target most of the metabolic derangements associated with metabolic diseases.

2.5 Garlic

Garlic is cultivated worldwide and is commonly used as a spice (Singh and Singh, 2008).

2.5.1 Taxonomy and botanical description

Garlic, scientifically known as *Allium sativum*, order Amaryllidales, family Alliaceae and genus Alieae (Gebreyohannes and Gebreyohannes, 2013). Its close relatives include the leek, onion, and shallot. Garlic has long leaves that resemble flat grass and its flowers are surrounded by a papery hood (Milner, 2005; Ameh *et al.*, 2013). Additionally, the flowers are clustered together at the end of a long stalk present with a pink or greenish-white colour (Ameh *et al.*, 2013).

2.5.2 Proximate, mineral, vitamin and phytochemical composition

Fresh garlic cloves have a water content of 65% (Suleria *et al.*, 2015). Dry garlic powder has 35% dry matter, 4.06% ash, 2% crude protein, and 0.6% lipid (Nwinuka *et al.*, 2005; Djankpa *et al.*, 2012; Suleria *et al.*, 2015). The major mineral in garlic includes selenium and germanium (Djankpa *et al.*, 2012). The powder contains phytochemicals largely in the form of organosulphur compounds. These organosulphur compounds largely made up of S-allyl cysteine, diallyl sulphide, ajoene and alliin (Garba *et al.*, 2013) constitute 2.3% of the garlic (Suleria *et al.*, 2015).

2.5.3 Uses of garlic

Garlic is one of the world-renowned spices used for culinary purposes (Suleria *et al.*, 2015). Traditionally it is used as an ethnomedicine in the management of ailments such as abdominal discomfort, respiratory tract infection and diarrhoea (Papu *et al.*, 2014). Garlic has been used as an antiseptic agent as demonstrated by garlic preparation that Pasteur used as antiseptic agents (Hosseini and Hosseinzadeh, 2015). Preparations of garlic are also used to treat wounds (Jalali *et*

al., 2009) and as prophylaxis and therapeutic agent (Asdaq, 2015). The use of garlic preparations in ethnomedicine hinges on its many biological properties that have health beneficial activities inclusive of anticancer, hypolipidaemic, hypoglycaemic, hypotensive, anti-atherosclerotic and anti-hyperinsulinemia (Elkayam *et al.*, 2003).

2.5.4 Phytochemical constituents

Phytochemicals are secondary plant metabolites that are produced to assist plants to protect themselves against herbivory and damage by pests (Bennett and Wallsgrove, 1994). In garlic, there is an array of these secondary bioactive compounds including among others saponins, alkaloids, tannins, glycosides and flavonoids (Ameh *et al.*, 2013; Garba *et al.*, 2013). The health beneficial properties of garlic are ascribed to various organosulfur compounds it contains (Yun *et al.*, 2014). The major organosulphur compounds in garlic include alliin, allicin, ajoene, diallyl disulfide, diallyl trisulfide, allyl methanethiosulfonate and S-allyl cysteine (Yun *et al.*, 2014).

2.5.5 Biological activities

Of the many health beneficial biological activities displayed by garlic preparations, it is their antiobesity, antioxidant, hypocholesterolaemic and hypoglycemic (Lee *et al.*, 2011) effects that could be exploited to mitigate high-fat and high-fructose diet-induced metabolic derangements and diseases (Joo *et al.*, 2013).

2.5.5.1 Antiobesity effects

Research has demonstrated that when garlic was administered to obese mice, it (garlic) reduced not only their body mass but also the mass of white adipose tissue (Lee *et al.*, 2011; Joo *et al.*, 2013). This physicochemical and pharmacological effect of garlic in regard of body mass and the mass of adipose tissue could be potentially exploited in studies that interrogate the prophylactic and therapeutic potential of garlic (Ameh *et al.*, 2013) especially in diet-induced obesity and its associated metabolic derangements and diseases. In obese mice, the consumption of 20ml of aqueous garlic extract in feed for 44 days was shown to lower serum lipid content (Djankpa *et*

al., 2012). The reduction in serum lipid content was ascribed to garlic extract's ability to reduce lipid absorption from the GIT and also its ability to reduce de novo hepatic cholesterol production (Djankpa *et al.*, 2012). An array of evidence has shown that raw garlic is effective in reducing hyperglycemia and attenuating many diabetic indicators, such as dyslipidemia and oxidative stress in both humans and animal models (Liu *et al.*, 2005; Thomson *et al.*, 2006; Drobiova *et al.*, 2011).

2.5.5.2 Antioxidant effects

High-fat high-fructose diet-driven derangements such as obesity and hyperglycemia result in lipid peroxidation which creates reactive oxygen species (ROS) that damage cell organelles, including cell membranes (Padiya *et al.*, 2011). This ROS-mediated oxidative damage occurs when ROS production exceeds the natural capacity of the body's antioxidant apparatus to mop up ROS. Increased ROS production requires enhancement of the body's antioxidant capacity in order to neutralize potential peroxidative damage. In male Sprague Dawley rats, garlic and garlic extracts have been shown to attenuate oxidative stress induced by the consumption of a high-fructose diet (Padiya *et al.*, 2011). Thus the need for determining osmotic fragility of erythrocyte to test for oxidative stress. Yin and Cheng (1998) observed that aqueous extracts of garlic had high antioxidant activities *in vitro*. It is suggested that the observed higher antioxidant activity displayed by garlic and/or its extracts arise from a cocktail of phytochemicals such as vitamins and phenolics which are known to have antioxidant activity (Yin and Cheng, 1998).

2.5.5.3 Hypocholesterolemic effects

One of the pathophysiological outcomes of deranged lipid metabolism is atherosclerosis (Bakker *et al.*, 2000). Orekhov and Grunwald (1997) reported that garlic prevents the onset and development of atherosclerotic lesions in animal models. Garlic induces regression of the lesions on artery walls through its anti-atherogenic or anti-atherosclerotic activity (Orekhov and Grunwald, 1997) which is exerted directly at cell (arterial) level in animal models. The efficacy of garlic at suppressing triglyceride and cholesterol ester biosynthesis in atherosclerotic cells has been demonstrated by a number of studies (Gebhardt and Beck, 1996; Yeh and Liu, 2001). Its

suppression of de novo lipid synthesis is mediated via the inhibition of Acyl-CoA: cholesterol acyltransferase (ACAT), an enzyme necessary for fat synthesis in cells (Londhe *et al.*, 2011).

2.5.5.4 Hypoglycaemic effects

In human and animal studies it has been demonstrated that garlic has anti-hyperglycaemic effects (Djankpa *et al.*, 2012). It has been postulated that garlic mediates hypoglycaemia through stimulating increased pancreatic insulin secretion by the pancreas and or through enhancing insulin sensitivity (El-Demerdash *et al.*, 2005). It is hypothesised that allicin, a bioactive compound found in garlic, could be the molecule behind garlic's stimulatory effects for increased insulin release from the pancreas (Londhe *et al.*, 2011).

While this chapter interrogated the literature pertinent to the study with a focus on obesity, metabolic derangements, fenofibrate as a pharmacological agent and garlic powder as an ethnomedicine, the next chapter is a detailed account of the materials and methods employed in the execution of the study.

CHAPTER 3: MATERIALS AND METHODS

3.0 Sourcing of garlic

Fresh garlic was purchased from the City Deep Market, Johannesburg, South Africa, in March 2016.

3.1 Preparation of garlic powder

The fresh garlic cloves were chopped and dried in an oven (Salvis®, Salvis Lab, Switzerland) at 40°C for 48 hours following which they were then ground to a fine powder in a coffee grinder (Russel Hobbs®, Failsworth, England). The resultant powder was put in a plastic container and stored in a dark cardboard at room temperature pending use.

3.2 Determination of the total phenolic content of garlic

The total phenolic content of the garlic powder was determined as described by Makkar. (2003). Briefly, the hydrophilic extract was produced by mixing 3g of the garlic powder with 25ml of methanol in a beaker followed homogenization. This homogenate was stored at 4°C for 12 hours then centrifuged (Hettich, Zentrifugen, Rotofix 32A, Baujahr, Germany) at 1699.36g for 20 minutes and the supernatant was stored at -20°C until analysis. To produce the lipophilic extract, the precipitate produced from the centrifugation process was homogenized in 70% acetone for and sonicated for 20 minutes then centrifuged (Hettich, Zentrifugen, Rotofix 32A, Germany) at 955.89g for 5 minutes. The resultant supernatant was stored at -20°C until analysis. To quantify the phenolics in the hydrophilic extract 150µl of the extract was added to a test tube containing 2.4ml of distilled water. One hundred and fifty (150) µl of Folin-Ciocalteu reagent solution (0.25N.) were added to the mixture which (mixture) was then thoroughly vortexed and then left for 3 minutes. Immediately thereafter 300µl of 5% sodium carbonate solution was added to the mixture, vortexed and incubated at room temperature for 2 hours in a dark cupboard. Absorbances were measured using a Beckman colter spectrophotometer (Beckman coulter DU® general purpose UV/Vis spectrophotometer) at 725nm. The assay was done in triplicate. A similar procedure was followed in determining the phenolic content in the lipophilic extract. A standard solution was prepared according to Thangaraj (2016). Briefly, a stock standard solution was prepared by dissolving 50mg of Gallic acid (GA) in methanol and making up to 50mL in a

flask. Thereafter, 5ml of the stock standard solution was diluted with 100ml of distilled water in a flask and the concentration of gallic acid in the working standard solution was 50µg/ml. Aliquots standard solution of 0.2, 0.4, 0.6, 0.8 and 1mL were pipetted into 5 test tubes respectively. The absorbances (y-axis) and concentration of gallic acid (x-axis) of the standard solution were used to plot a standard graph (calibration curve). The phenolic content in the hydrophilic and lipophilic extracts was then determined by reading off the concentration of the phenolics from the standard/calibration curve.

The total phenolic content of garlic powder was calculated using the formula below:

$C = cV/m$

C- total phenolic content (mg GAE/g dry extract)

c- concentration of gallic acid obtained from the calibration curve (mg/ml)

V- the volume of extract (ml)

m- a mass of extract (g)

3.3 Ethical clearance and the study site

Ethical approval for the use of animals in this study was applied and obtained from the Animal Ethics Screening Committee (AESC) of the University of the Witwatersrand (AESC number: 2016/03/13B). The study was conducted in the Central Animal Services facility and the School of Physiology laboratories at the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.

3.4 Animals, feeding, and housing

Twenty-one day old Sprague Dawley rats were used in the study. The rats were housed individually in standard Perspex rat cages with clean wood shavings and shredded paper for bedding. The bedding was changed twice a week. Room temperature was maintained at 24±2°C with a 12h light-dark cycle (lights on from 7 am to 7 pm). Each rat had *ad libitum* access to feed (either a standard rat chow (SRC) and/or SRC supplemented with beef tallow at 2% (w/w) and plain drinking water or fructose solution of 20% (w/v), depending on the treatment regimen.

3.5 Experimental design

Eighty 21-days old Sprague Dawley rats (40 male and 40 female) were habituated for 2 days and then randomly (10 males and 10 females) allocated to and administered one of the following treatment regimens: group 1: standard rat chow (SRC) + plain drinking water (PDW) + plain gelatine cube (PGC); group 2: SRC supplemented with beef tallow [SRCB; 2% (w/w)] + 20% fructose solution (FDW) + plain gelatine cube (PGC); group 3: SRCB + FDW + gelatine cube containing fenofibrate (FGC) at 100mg/kg body mass/day and group 4: SRCB + FDW + gelatine cube containing garlic powder (GGC) at 100mg/kg body mass/day. The dosage of fenofibrate and garlic powder used in the current study were similar to those used by Barnard *et al.* (2009) and Fallon *et al.* (1998) respectively. The gelatine cubes used as vehicles to administer fenofibrate and garlic powder were prepared as previously described by Kamerman *et al.* (2004). The rats were maintained on the respective treatment regimens for 8 weeks.

3.6 Measurements

3.6.1 Body mass measurement

The rats were weighed using an electronic balance (Snowrex Electronic Scale, Clover Scale (Pty) Ltd, Johannesburg, South Africa) twice a week to monitor growth performance and to maintain a constant dose of the fenofibrate and/or garlic during the 8-week treatment period.

3.6.2 Oral glucose tolerance test

On a postnatal day 79, the rats were fasted overnight following which fasting blood glucose concentration was determined. Blood for determining fasting glucose concentration was drawn from the tail vein following sterilization of the tail and a pin-prick (Thangaraj, 2016). A calibrated Contour-plus glucometer (Contour Plus®, Bayer Corporation, and Mishawaka, USA) was used to measure the glucose concentration in the blood. Following the determination of

basal glucose concentration, the rats were then each orally gavaged with 2g/kg body mass of sterile 50% (w/v) D- (+)-glucose solution [Merck Chemicals (Pty) Ltd, Johannesburg, South Africa]. Post-gavage blood glucose concentration was then determined at time intervals 15, 30, 60 and 120 minutes (Thangaraj, 2016).

3.7 Terminal procedures

Following the OGTT, the rats were returned to their respective treatment regimens for 48 hours and allowed to recover. Thereafter the rats were fasted overnight following which blood glucose concentration was determined using a calibrated glucometer (Contour Plus®, Bayer Corporation, Mishawaka, USA) and blood triglyceride concentration was also determined using a calibrated Accutrend GCT meter (Accutrend, Roche Company, Mannheim, Germany) as per the manufacturer's instructions. The rats were then euthanized with an overdose of sodium pentobarbitone (Euthanaze, Centaur labs, Johannesburg, South Africa) injected intra-peritoneal at a dose of 150mg/kg per body mass.

3.7.1 Blood collection, processing, and plasma storage

After euthanasia, blood was collected from each rat via cardiac puncture into 8ml heparinised blood collection tubes (BD Vacutainer™ LH Becton, Dickinson and company, Belliver Industrial Estate, UK) using 21G needles and 10ml syringes. The collected blood samples were centrifuged at 5000g for 15 minutes at 20^0C (Sorvall RT® 6000B, Pegasus Scientific Inc., Rockville, USA). The collected plasma, in micro-tubes, was then stored at -20^0C pending further assays.

3.7.2 Determination of packed cell volume

A small portion of the collected blood was separated into micro-capillary tubes and then used to determine packed cell volume (PCV) by centrifuging at 3500g for 2 minutes in a micro-haematocrit centrifuge (IDEXX Statspin® VT Centrifuge, Iris Sample Processing USA).

3.7.3 Determination of erythrocyte osmotic fragility

Erythrocyte osmotic fragility was determined as described by (Moyo *et al.*, 2012). Fifty microlitres of blood collected from each rat was dispensed into a test tube containing 5ml of phosphate-buffered saline (pH 7.4) with serial saline concentrations ranging from 0% to 0.85%. The contents of each tube were mixed and allowed to incubate at room temperature (approximately 27°C) for 30 minutes following which they were then centrifuged at 5000g for l5 minutes. The supernatants were decanted into cuvettes and absorbances read using a spectrophotometer (Beckman coulter DU® general purpose UV/Vis spectrophotometer) at 540nm. Distilled water was used as a blank. The percentage of haemoglobin released was calculated for each rat. Complete haemolysis was assumed from a concentration that had the highest absorbance. The percentage haemoglobin release was used to plot fragilograms.

3.7.4 Determination of viscera macro-morphometry

Following blood collection, a midline incision was made on each rat carcass and viscera (liver, pancreas, small and large intestines, visceral fat, kidneys, and epididymal fat) were carefully dissected out and weighed on an electronic balance (Presica 310M electronic balance, Presica Instruments AG, Switzerland). The lengths of the small and large intestines were determined by gently stretching them on a ruler attached to the dissecting board. The kidneys and a sample of the liver were preserved in 10% phosphate-buffered formalin for histology. The remainders of the liver were stored at -20°C refrigerator for later determination of liver lipid content. The mass of the empty carcass for each rat was then measured on the Presica electronic balance. The carcasses were freeze stored pending the determination of linear growth.

3.7.5 Determination of linear growth

The femoral attachment of the right hind leg to the pelvis was meticulously dissected out from each rat carcass and then disarticulated from the tibia and defleshed. After defleshing, the femora

and tibiae were dried to constant mass in an oven (Salvis®, Salvis Lab, Switzerland) at 50°C for 5 days and their mass was determined using an electronic balance (Presica 310M electronic balance, Presica Instruments AG, Switzerland). Thereafter the tibia length measured between tibia head and medial malleolus and femur length, measured between distal femoral articular surfaces to the greater trochanter, were measured with a digital calliper (KTV150 Digital Vernier Calliper, Major Tech (PTY) LTD, Elandsfontein, South Africa). The bone mass to bone length ratio was then calculated using the formula:

bone mass to bone length $= \frac{\text{mass of bone (mg)}}{\text{length of bone (mm)}}$ as described by (Seedor *et al.*, 1991)

3.7.6 Determination of liver lipid content

The liver lipid content was determined using the Soxtec Apparatus as described by the Association of Analytical Chemists (AOAC, 2005, method number 920.39).

3.7.7 Determination of liver and kidney micro-morphometry

The liver and kidneys preserved in formalin were used in the determination of micro-morphometry. Each tissue sample was embedded in paraffin wax and sectioned using a microtome (Leica Biosystems, Nussloch, Heidelberger, Germany). The sections were mounted on a glass slide, stained with haematoxylin and eosin and then covered with a glass cover slip. The slides were then viewed under a light microscope (Reichert®, Austria). Using a high power magnification of 400X, the size of hepatocytes and the number of cells within a linear field (100µm) were measured. The haematoxylin and eosin-stained sections of the liver were semi-quantitatively scored for steatosis, inflammation and hepatocellular ballooning as described by Kleiner *et al.* (2005). Steatosis was determined by analysing hepatocellular vesicular steatosis based on the total area affected. Grading for steatosis was done based on the following criteria: grade 0 ≤ 5% steatosis, grade 1 = 5-33% steatosis; grade 2 = 33-66% steatosis and grade 3 ≥ 66% steatosis per camera field of the liver parenchyma (Brunt *et al.*, 1999; Kleiner *et al.*, 2005). Inflammation was scored by counting the number of inflammatory cell aggregates in the liver

parenchyma and graded according to the following criteria: grade 0 = none or no foci of inflammation per camera field, grade 1 = fewer than 2 foci per camera field, grade 2 = 2 - 4 foci per camera field and grade 3 ≥ 4 foci per camera field. The scoring was done at ×20 magnification. Ballooning was evaluated using three categories namely: none, few, and/or many. Renal corpuscular and glomerular area measurements were quantitatively assessed from photomicrographs of haematoxylin and eosin stained sections using ImageJ software at ×40 magnification as described by Schneider *et al.* (2012).

3.7.8 Determination of surrogate markers of liver and kidney function

Plasma activities of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and plasma creatinine, urea, and cholesterol concentration of the rats were determined using an IDEXX colorimetric-based clinical chemistry analyser (IDEXX Catalyst Dx Clinical Chemistry Analyser, IDEXX Laboratories Inc., USA) as per the manufacturer's instructions. Briefly, each plasma sample was thawed and allowed to warm to room temperature and then gently inverted to mix the contents. The plasma sample was then placed into the analyser which automatically drew up 300µL of the plasma. Ten microliters (10µL) of plasma were then loaded onto each of the pre-loaded disks after which each sample was then analysed and the results were displayed on the screen.

3.7.9 Determination of plasma insulin concentration

The plasma insulin concentration was determined using an enzyme-linked immunosorbent assay (ELISA) kit with a Rat Insulin Kit (Elabscience Biotechnology Inc. OCBC Center, Singapore) as per the manufacturer's instructions. The assay employed a quantitative sandwich enzyme immunoassay technique which utilizes monoclonal antibodies specific for rat insulin. Absorbencies of the plasma were read at 450 nm using a microplate reader (Elabscience Biotechnology Inc. OCBC Center, Singapore). A standard curve was constructed using calibrator concentration. The concentrations of insulin in the samples were determined from the standard

curve. A detailed description of the protocol is shown in appendix 2. The Elisa kit had a detection range of 3.13-200ng/ml.

3.7.10 Computation of the HOMA-IR index

Fasting whole-body insulin sensitivity was computed using the homeostasis model assessment of insulin resistance using the equation described by Matthews *et al.* (1985).

HOMA-IR = [fasting insulin (ng/mL) × fasting glucose (mg/dl)/405]

3.8 Statistical analysis

Parametric data are expressed as mean ± SD while non-parametric data are expressed as median and range (minimum, maximum). Data analysis was done using GraphPad Prism 5 software (Graph-pad Software Inc., San Diego, USA). The weekly body masses and OGTT data within groups were analysed using a repeated measure analysis of variance (ANOVA) while other parametric data of various groups were analysed using a one-way ANOVA. A Bonferroni post hoc test was used to compare the means. Data on non-alcoholic fatty liver disease score (NAS) was analysed using the Kruskal-Wallis test followed by the Dunns post hoc test that compared the medians. Statistical significance was considered when $P < 0.05$.

The following chapter presents the results obtained from this study.

CHAPTER 4: RESULTS

4.0 Phenolic content of garlic powder

The hydrophilic and lipophilic extract's phenolic content of garlic powder was 0.022mgGAE/g and 0.06mgGAE/g respectively, thus the total phenolic content of garlic powder was 0.082mgGAE/g.

4.1 Growth performance

4.1.1 Body mass

Figure 4.1 below shows the induction and terminal body masses of the male rats. While the induction body masses of the rats were similar, the rats grew significantly ($P < 0.001$) during the experimental period. Despite the significant growth observed during the experimental period, the terminal body masses of the male rats were similar ($P > 0.05$) across treatment regimens.

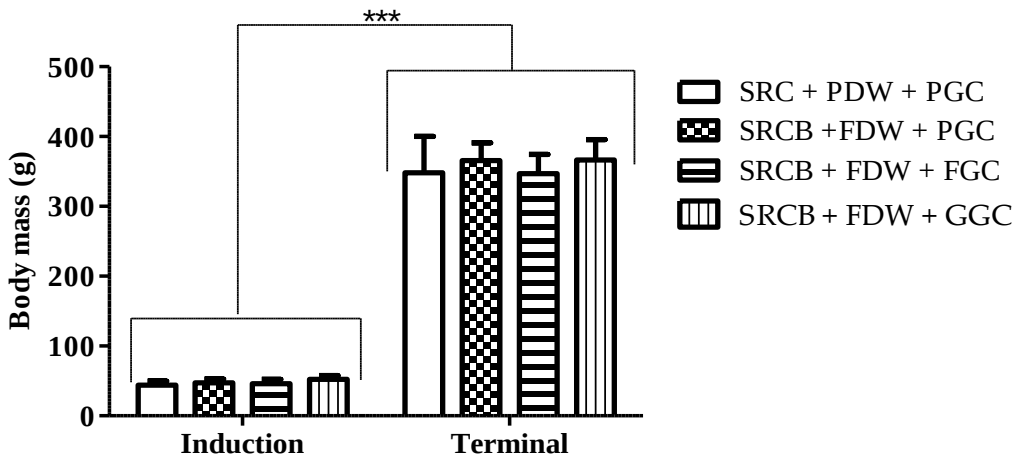


Figure 4.1: The induction and terminal body masses of the male rats.

*** $P < 0.001$. The induction body masses of the rats were similar. Terminal body masses of the rats were heavier compared to the induction body masses but were similar across treatment regimens. SRC + PDW + PGC = standard rat chow + plain drinking water + plain gelatine cube; SRCB + FDW + PGC = standard rat chow with 2% beef tallow (w/w) + 20 % fructose in drinking water (w/v) + plain gelatine cube; SRCB + FDW + FGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + fenofibrate gelatine cube (100 mg/kg body mass/day); SRCB + FDW + GGC= standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + garlic powder gelatine cube (100 mg/kg body mass/day); Data presented as mean ± SD; n =10 per treatment.

The induction and terminal body masses of the female rats are shown in figure 4.2 below. Albeit the similarity in the induction body masses of the rats, the rats grew significantly ($P < 0.001$) during the experimental period. Apart from the significant growth of the rats during the experimental period, the terminal body masses of the rats were similar ($P > 0.05$) across treatment regimens.

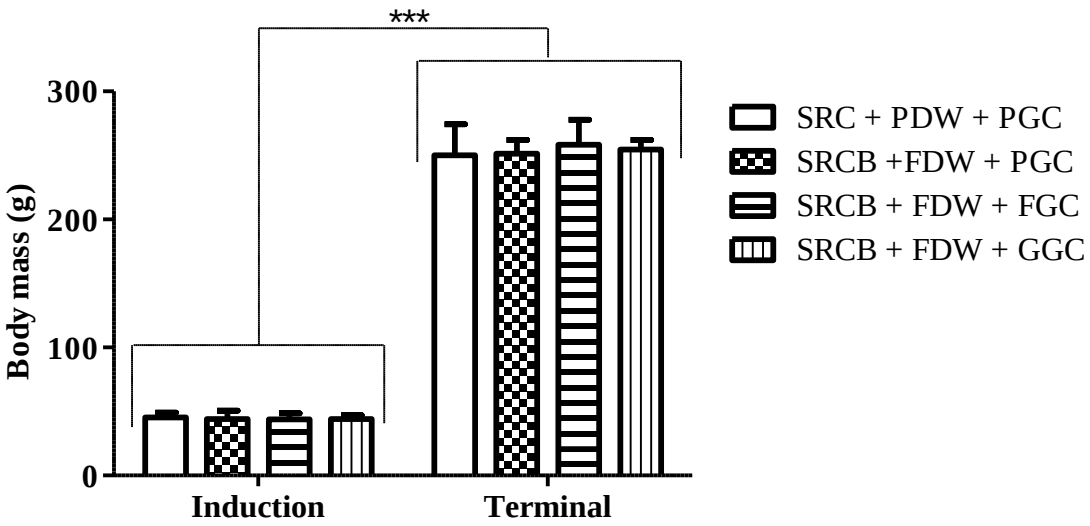


Figure 4.2: The induction and terminal body masses of the female rats.

***P < 0.001. The induction body masses of the rats were similar. Although terminal body masses of these rats were higher than the induction body masses, they were statistically similar across treatment regimens. SRC + PDW + PGC = standard rat chow + plain drinking water + plain gelatine cube; SRCB + FDW + PGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + plain gelatine cube; SRCB + FDW + FGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + fenofibrate gelatine cube (100 mg/kg body mass/day); SRCB + FDW + GGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + garlic powder gelatine cube (100 mg/kg body mass/day); Data presented as mean ± SD; n =10 per treatment.

4.1.2 Linear growth

The mass, length and Seedor ratio of the tibiae and femora of the male rats across treatment regimens is presented in table 4.1 below. The tibia and femora masses, lengths and Seedor ratio of the male rats to which the four treatment regimens were administered were similar ($P > 0.05$).

Table 4.2 below presents the mass, length and Seedor ratio of the tibiae and femora of female rats across treatment regimens. The masses, lengths and Seedor ratio of tibia and femora of female rats from which the four treatment regimens were administered were similar ($P > 0.05$).

Table 4.1: Effect of garlic powder on tibiae and femora masses, lengths and Seedor ratio from the male rats fed a high-fat high-fructose diet

Parameters	SRC + PDW + PGC	SRCB + FDW + PGC	SRCB + FDW + FGC	SRCB + FDW + GGC	Significance
Tibia					
Mass (mg)	565.60 ± 44.79[a]	557.20 ± 38.72[a]	579.40 ± 38.70[a]	564.70 ± 43.15[a]	ns
Length (mm)	42.68 ± 1.59[a]	42.95 ± 1.48[a]	42.98 ± 1.69[a]	42.47 ± 1.80[a]	ns
Seedor index (mg/mm)	13.25 ± 0.95[a]	12.97 ± 0.70[a]	13.48 ± 0.97[a]	13.31 ± 0.91[a]	ns
Femur					
Mass (mg)	700.30 ± 55.99[a]	697.40 ± 36.25[a]	682.30 ± 36.80[a]	683.60 ± 60.20[a]	ns
Length (mm)	35.57 ± 1.26[a]	35.12 ± 0.68[a]	34.62 ± 0.71[a]	34.77 ± 0.87[a]	ns
Seedor index (mg/mm)	19.67 ± 1.05[a]	19.85 ± 0.74[a]	19.71 ± 0.99[a]	19.64 ± 1.43[a]	ns

n.s = not significant, [a]Within rows means with similar superscripts are not significantly different ($P > 0.05$). The tibiae and femora masses, lengths and Seedor ratio were similar across treatment regimens. SRC + PDW + PGC = standard rat chow + plain drinking water + plain gelatine cube; SRCB + FDW + PGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + plain gelatine cube; SRCB + FDW + FGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + fenofibrate gelatine cube (100 mg/kg body mass/day); SRCB + FDW + GGC= standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + garlic powder gelatine cube (100 mg/kg body mass/day). Data presented as mean ± SD; n =10 per treatment

Table 4.2: Effect of garlic powder on tibiae and femora masses, lengths and Seedor ratio from female rats fed a high-fat high-fructose diet

Parameters	SRC +PDW + PGC	SRCB + FDW + PGC	SRCB + FDW + FGC	SRCB + FDW + GGC	Significance
Tibia					
Mass (mg)	482.40 ± 18.57[a]	468.60 ± 20.69[a]	477.00 ± 21.59[a]	472.70 ± 16.91[a]	ns
Length (mm)	39.63 ± 1.16[a]	39.77 ± 1.63[a]	39.27 ± 1.04[a]	40.18 ± 0.98[a]	ns
Seedor index (mg/mm)	12.17 ± 0.60[a]	11.79 ± 0.51[a]	12.15 ± 0.60[a]	11.76 ± 0.31[a]	ns
Femur					
Mass (mg)	601.40 ± 32.01[a]	578.10 ± 31.94[a]	582.70 ± 40.74[a]	575.30 ± 21.33[a]	ns
Length (mm)	33.54 ± 1.31[a]	32.74 ± 0.85[a]	32.71 ± 0.74[a]	32.89 ± 0.49[a]	ns
Seedor index (mg/mm)	17.98 ± 1.03[a]	17.56 ± 1.03[a]	17.81 ± 0.97[a]	17.45 ± 0.64[a]	ns

n.s = not significant, [a]Within rows means with similar superscripts are not significantly different ($P > 0.05$). The masses, lengths and Seedor ration of both tibiae and femora of the rats were similar across treatment regimens. SRC + PDW + PGC = standard rat chow + plain drinking water + plain gelatine cube; SRCB + FDW + PGC = standard rat chow with 2% beef tallow (w/w) + 20 % fructose in drinking water (w/v) + plain gelatine cube; SRCB + FDW + FGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + fenofibrate gelatine cube (100 mg/kg body mass/day); SRCB + FDW + GGC= standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + garlic powder gelatine cube (100 mg/kg body mass/day). Data presented as mean ± SD; n =10 per treatment.

4.2 Haematological parameters

4.2.1 Packed cell volume

Figure 4.3 illustrates the effect of garlic powder on the packed cell volume of the male rats fed a high-fat high-fructose diet. The PCV of the male rats to which the control treatment regimen was administered was significantly higher compared to the PCV of their counterparts to which the test treatment regimens were administered.

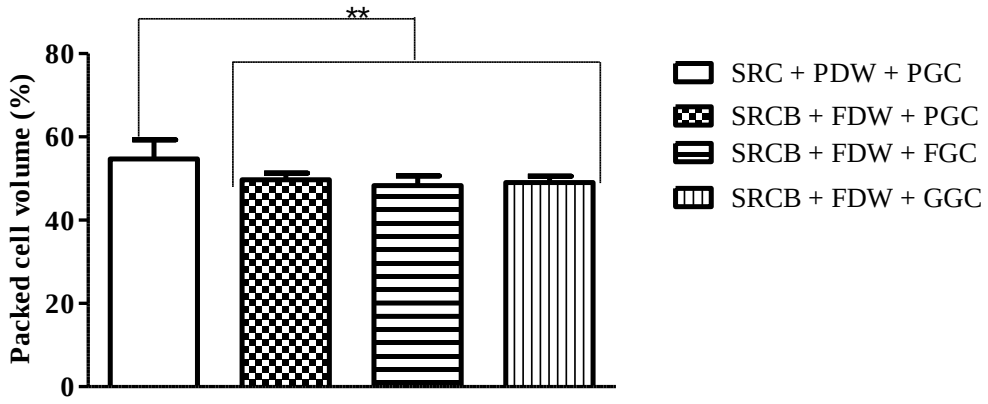


Figure 4.3: Effect of garlic powder on the packed cell volume of the male rats fed a high-fat high-fructose diet.

** $P < 0.01$. The male rats to which the control treatment regimen was administered had a significantly higher ($P < 0.05$) packed cell volume compared to that of their counterparts administered other treatment regimens. SRC + PDW + PGC = standard rat chow (SRC) + plain drinking water (PDW) + plain gelatine cube (PGC); SRCB + FDW + PGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + plain gelatine cube; SRCB + FDW + FGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + fenofibrate gelatine cube (100 mg/kg body mass/day); SRCB + FDW + GGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + garlic powder gelatine cube (100 mg/kg body mass/day). Data presented as mean ± SD; n =10 per treatment

Figure 4.4 below shows the effect of garlic powder on the packed cell volume of the female rats fed a high-fat high-fructose diet. The PCV of the female rats to which the four treatment regimens were administered were statistically similar (P = 0.4691).

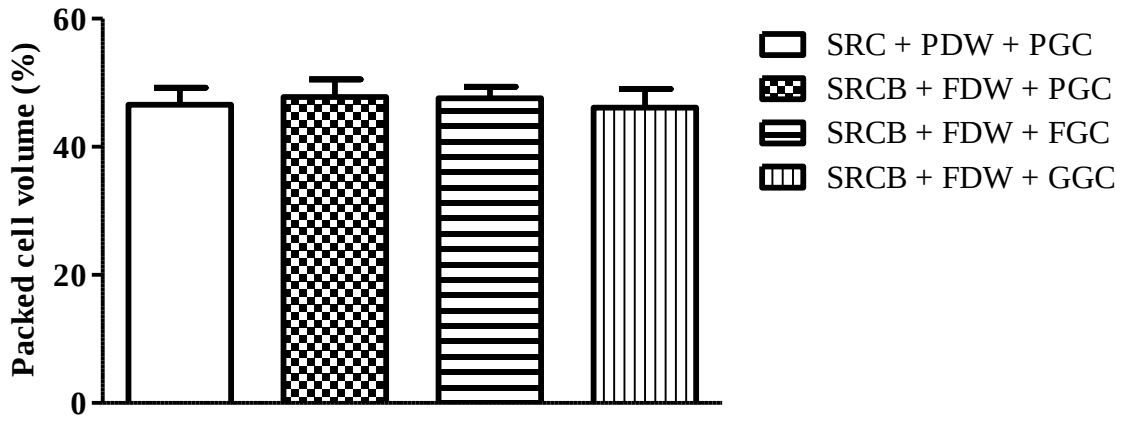


Figure 4.4: Effect of garlic powder on the packed cell volume of the female rats fed a high-fat high-fructose diet.

The packed cell volumes of rats were similar (P = 0.4691) across treatment regimens. SRC + PDW + PGC = standard rat chow + plain drinking water + plain gelatine cube; SRCB + FDW + PGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + plain gelatine cube; SRCB + FDW + FGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + fenofibrate gelatine cube (100 mg/kg body mass/day); SRCB + FDW + GGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + garlic powder gelatine cube (100 mg/kg body mass/day). Data presented as mean ± SD; n =10 per treatment.

4.2.2 Erythrocyte osmotic fragility

The fragilogram of erythrocytes of the male rats is shown in figure 4.5 below. The osmotic fragility of erythrocytes in male rats was similar (P > 0.05) across treatment regimens. The mean corpuscular fragility (MCF) (50% haemolysis) occurred between 0.4 – 0.5% PBS concentration.

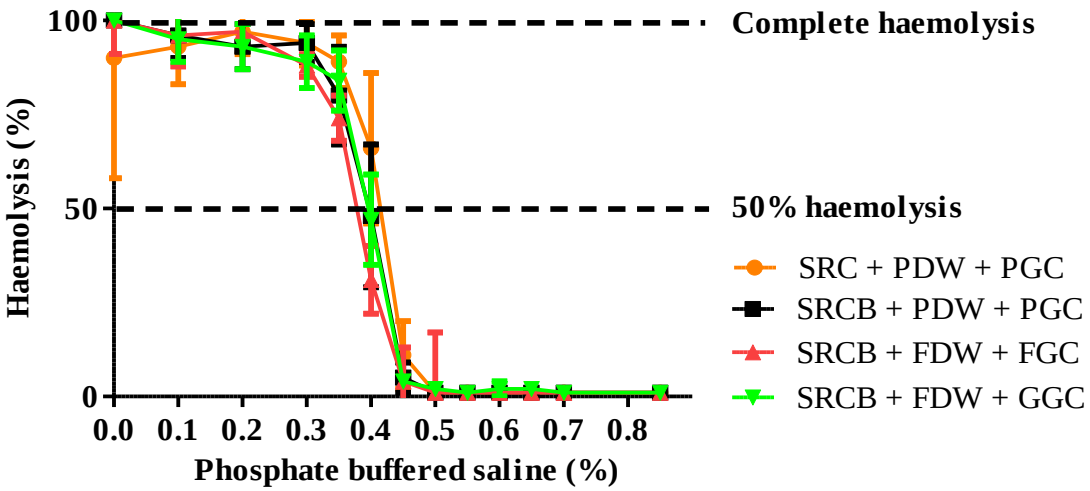


Figure 4.5: Effect of garlic powder on the haemolysis of erythrocytes of the male rats fed a high-fat high-fructose diet.

The rats' mean erythrocyte haemolysis was similar across treatment regimens. SRC + PDW + PGC = standard rat chow (SRC) + plain drinking water (PDW) + plain gelatine cube (PGC); SRCB + FDW + PGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + plain gelatine cube; SRCB + FDW + FGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + fenofibrate gelatine cube (100 mg/kg body mass/day); SRCB + FDW + GGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + garlic powder gelatine cube (100 mg/kg body mass/day). Data presented as means; n =10 per treatment.

The fragilogram of erythrocytes for the female rats is shown in figure 4.6 below. The erythrocyte osmotic fragility of female rats was similar (P > 0.05) across treatment regimens. The mean corpuscular fragility (MCF) (50% haemolysis) occurred at 0.4 – 0.5% PBS concentration.

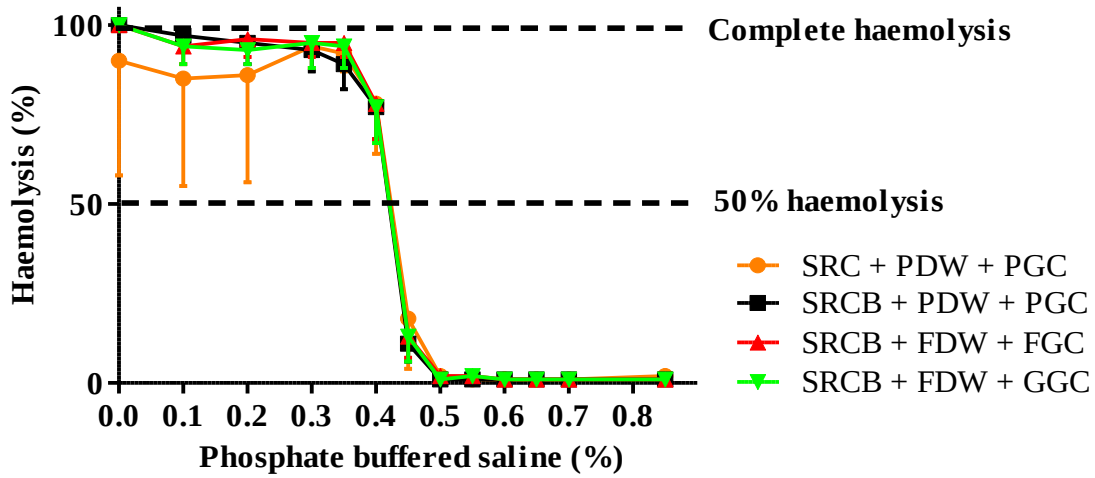


Figure 4.6: Effect of garlic powder on the haemolysis of erythrocytes of female rats fed a high-fat high-fructose diet.

Rats across treatment regimens had similar ($P > 0.05$) mean erythrocyte haemolysis. SRC + PDW + PGC = standard rat chow + plain drinking water + plain gelatine cube; SRCB + FDW + PGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + plain gelatine cube; SRCB + FDW + FGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + fenofibrate gelatine cube (100 mg/kg body mass/day); SRCB + FDW + GGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + garlic powder gelatine cube (100 mg/kg body mass/day). Data presented as means; n =10 per treatment.

4.2.3 Tolerance to glucose load

The blood glucose concentration of the male rats following a challenge by an oral glucose load is shown in figure 4.7 below. While the blood glucose concentration of the male rats to which the control and a high-fat high-fructose diet treatment regimens were administered significantly peaked ($P < 0.001$) at 30 minutes post gavage, that of the rats to which the positive control (with fenofibrate) and intervention (with garlic powder) peaked ($P < 0.001$) at 15 minutes' post-gavage. Across treatment regimens, the blood glucose concentration of the male rats returned to the basal concentration at 120 minutes post-gavage.

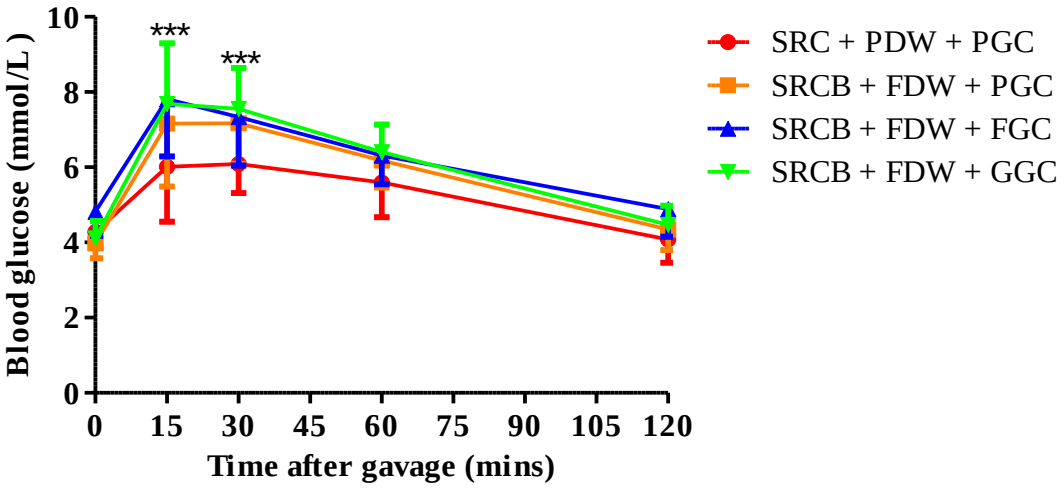


Figure 4.7: Effect of garlic powder to tolerance of an oral glucose load by male rats fed a high-fat high-fructose diet.

***P < 0.001. Blood glucose concentration of the rats fed the control, high-fat high-fructose diet treatment regimens peaked 30 minutes post gavage while that of the rats fed the positive control (with fenofibrate) and intervention (with garlic powder) treatment regimens peaked 15 minutes' post gavage. The blood glucose concentration of the rats across treatment regimens returned to the basal concentration by 120 minutes post gavage. SRC + PDW + PGC = standard rat chow (SRC) + plain drinking water (PDW) + plain gelatine cube (PGC); SRCB + FDW + PGC = standard rat chow with 2% beef tallow (w/w) + 20 % fructose in drinking water (w/v) + plain gelatine cube; SRCB + FDW + FGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + fenofibrate gelatine cube (100 mg/kg body mass/day); SRCB + FDW + GGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + garlic powder gelatine cube (100 mg/kg body mass/day). Data presented as mean ± SD; n =10 per treatment.

The blood glucose concentration of the female rats following a challenge by an oral glucose load is presented in figure 4.8 below. The blood glucose concentration of the female rats to which the control, high-fat high-fructose diet and positive control (with fenofibrate) treatment regimens were administered significantly peaked (P < 0.001) at 15 minutes post gavage while that of the rats to which the intervention (with garlic powder) treatment regimen was administered significantly peaked (P < 0.001) at 30 minutes post-gavage. The blood concentration of the

female rats across treatment regimen returned to basal concentration by 120 minutes post-gavage.

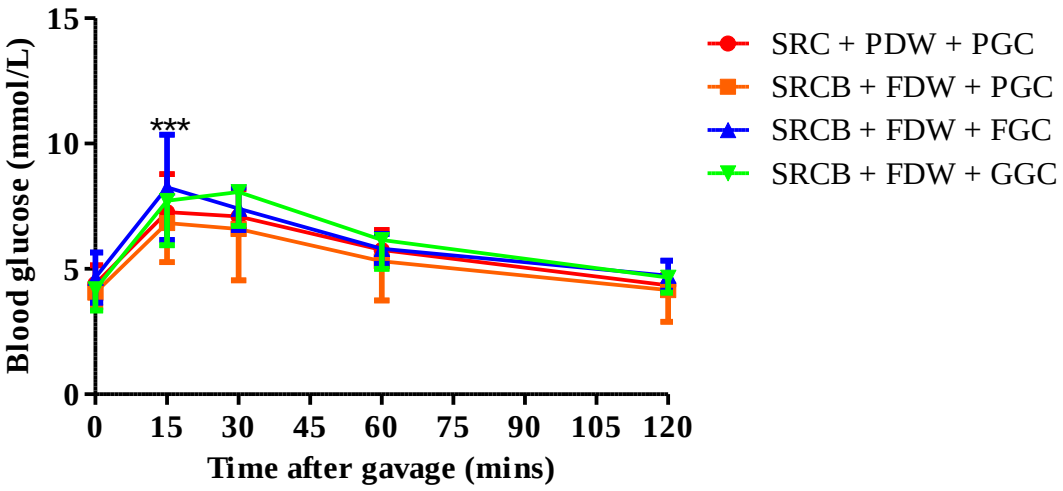


Figure 4.8: Effect of garlic powder to tolerance of an oral glucose load by female rats fed a high-fat high-fructose diet.

***P < 0.001. Blood glucose concentration of the rats fed control and a high-fat high-fructose diet and that of rats fed the positive control (with fenofibrate) peaked at 15 minutes post gavage while that of the rats on an intervention (with garlic powder) treatment regimen peaked at 30 minutes post gavage. The blood glucose concentration of the rats across treatment regimens returned to the basal concentration by 120 minutes post gavage. SRC + PDW + PGC = standard rat chow (SRC) + plain drinking water (PDW) + plain gelatine cube (PGC); SRCB + FDW + PGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + plain gelatine cube; SRCB + FDW + FGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + fenofibrate gelatine cube (100 mg/kg body mass/day); SRCB + FDW + GGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + garlic powder gelatine cube (100 mg/kg body mass/day). Data presented as mean ± SD; n =10 per treatment.

4.2.4 The area under the curve

The area under the curve of the glucose tolerance test of the male rats is shown in figure 4.9 below. Following a challenge by an oral glucose load, the areas under the curve of male rats to which the positive control (with fenofibrate) and intervention (with garlic powder) treatment regimens were administered were significantly higher (P = 0.0125) compared to that of the male rats fed the control and the high-fat high-fructose diet treatment regimens.

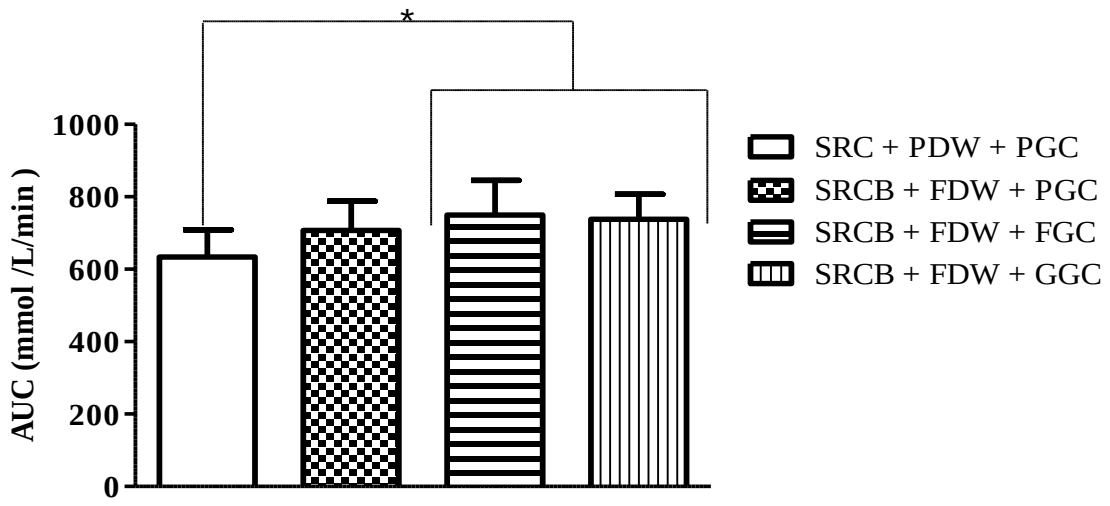


Figure 4.9: Effect of garlic powder on the area under the curve following a challenge by an oral glucose load in male rats fed a high-fat high-fructose diet.

*P < 0.05. The area under the curve for rats that had fenofibrate (positive control) and garlic powder (test intervention) was significantly higher (P < 0.05) compared to that of rats fed the control and high-fat high-fructose diet. SRC + PDW + PGC = standard rat chow + plain drinking water + plain gelatine cube; SRCB + FDW + PGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + plain gelatine cube; SRCB + FDW + FGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + fenofibrate gelatine cube (100 mg/kg body mass/day); SRCB + FDW + GGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + garlic powder gelatine cube (100 mg/kg body mass/day). Data presented as mean ± SD; n =10 per treatment.

The area under the curve of the glucose tolerance test of the female rats is shown in figure 4.10 below. The area under the curve of the female rats following a challenge by an oral glucose load was similar (P > 0.05) across treatment regimens.

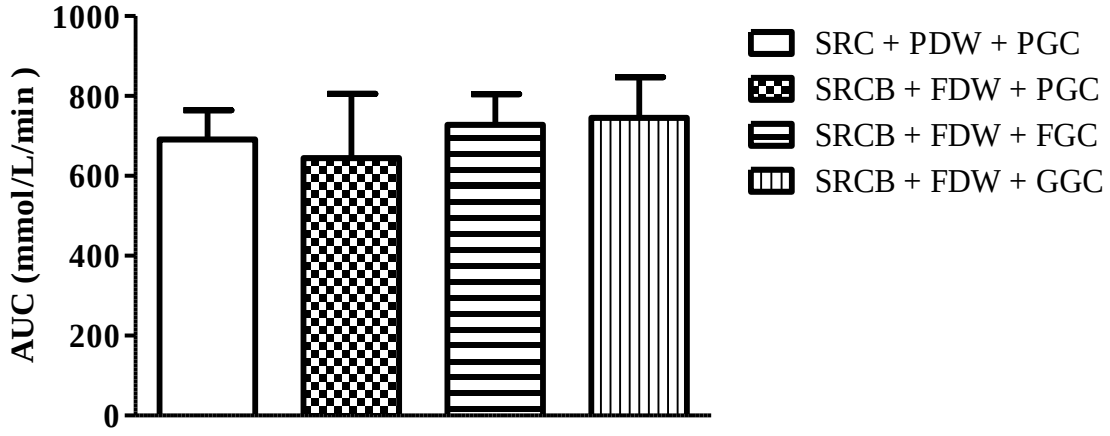


Figure 4.10: Effect of garlic powder on the area under the curve following a challenge by an oral glucose load in female rats fed a high-fat high-fructose diet.

No significant differences (P > 0.05) in the area under the curve of the female rats were observed. SRC + PDW + PGC = standard rat chow + plain drinking water + plain gelatine cube; SRCB + FDW + PGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + plain gelatine cube; SRCB + FDW + FGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + fenofibrate gelatine cube (100 mg/kg body mass/day); SRCB + FDW + GGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + garlic powder gelatine cube (100 mg/kg body mass/day). Data presented as mean ± SD; n =10 per treatment.

4.3 Circulating and hepatic metabolites

Table 4.3 below shows the blood glucose and insulin concentration, HOMA-IR index, liver lipid content, plasma triglyceride, and cholesterol concentration in male rats. The male rats to which the high-fat high-fructose diet and garlic powder as an intervention were administered had significantly higher ($P = 0.0007$) fasting blood triglyceride concentration compared to their counterparts to which the control treatment regimen was administered. There were no significant differences ($P > 0.05$) in the blood glucose, cholesterol and insulin concentration as well as the HOMA-IR indices of the male rats across treatment regimens. The male rats to which high-fat high-fructose diet was administered had significantly lower ($P < 0.001$) liver lipid content compared to their counterparts to which the control, fenofibrate and garlic powder were administered. The liver lipid content of rats to which the high-fat high-fructose diet with garlic powder and/or fenofibrate as interventions were administered was similar but significantly higher compared to the liver lipid content of the rats to which the control diet was administered.

Table 4.3: Effect of garlic powder on blood glucose and plasma insulin concentration, HOMA-IR index, liver lipid content, triglyceride and cholesterol concentration of male rats fed a high-fat high-fructose diet

Parameters	SRC + PDW + PGC	SRCB + FDW + PGC	SRCB + FDW + FGC	SRCB + FDW + GGC	Significance
Glucose (mg/dL)	65.52 ± 8.74[a]	65.52 ± 9.98[a]	76.86 ± 8.19[a]	66.42 ± 9.91[a]	ns
Insulin (ng/mL)	50.37 ± 16.93[a]	40.85 ± 12.45[a]	47.32 ± 14.33[a]	45.18 ± 20.90[a]	ns
HOMA-IR	8.31 ± 3.28[a]	6.70 ± 2.41[a]	8.95 ± 2.55[a]	7.30 ± 3.03[a]	ns
Liver lipid content (%)	2.54 ± 0.08[a]	2.04 ± 0.15[b]	2.79 ± 0.09[c]	2.75 ± 0.23[c]	***
Triglyceride (mmol/L)	1.31 ± 0.30[a]	1.95 ± 0.31[b]	1.77 ± 0.48[ab]	2.23 ± 0.66[b]	*
Cholesterol (mmol/L)	1.03 ± 0.17[a]	1.05 ± 0.14[a]	1.00 ± 0.14[a]	1.15 ± 0.14[a]	ns

n.s = not significant, ***P < 0.001; *P < 0.05. [ab]Within row means with different superscripts are significantly different at P < 0.05. Blood glucose and plasma insulin concentration, HOMA-IR and cholesterol concentration of the rats were similar (P > 0.05) across treatment regimens. The liver lipid content of rats to which the treatment regimens with fenofibrate and garlic powder were administered was significantly higher (P < 0.001) compared to that of counterparts to which the control and high-fat high-fructose treatment regimens were administered. Rats fed a high-fat high-fructose diet had significantly lower (P < 0.001) liver lipid content compared to those fed the control treatment regimen. The triglyceride concentration of rats to which high-fat high-fructose diet and intervention with garlic powder treatment regimens were administered were significantly higher than that of rats fed the control diet. SRC + PDW + PGC = standard rat chow + plain drinking water + plain gelatine cube; SRCB + FDW + PGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + plain gelatine cube; SRCB + FDW + FGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + fenofibrate gelatine cube (100 mg/kg body mass/day); SRCB + FDW + GGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + garlic powder gelatine cube (100 mg/kg body mass/day); HOMA-IR = homeostatic model of insulin resistance. Data presented as mean ± SD; n =7-10 per treatment.

The blood glucose and plasma insulin concentration, HOMA-IR index, liver lipid content, triglyceride, and cholesterol concentration of the female rats are shown in table 4.4 below. The female rats to which fenofibrate as an intervention was administered had significantly higher ($P = 0.0055$) fasting blood glucose concentration compared to that of their counterparts to which the control and high-fat high-fructose diets were fed. The plasma triglyceride concentration of rats to which a high-fat high-fructose diet (HFHF) and HFHF with either fenofibrate or garlic powder as interventions was significantly higher ($P = 0.0004$) than that of rats to which the control diet was fed. There were no significant differences ($P > 0.05$) in the insulin and cholesterol concentrations as well as the HOMA-IR indices of the female rats across the treatment regimens. The liver lipid content of the rats fed the HFHF diet with either fenofibrate or garlic powder as interventions was significantly higher ($P < 0.05$) compared to that of rats fed the control diet or the HFHF diet.

Table 4.4: Effect of garlic powder on blood glucose and plasma insulin concentration, HOMA-IR index, liver lipid content, plasma triglyceride and cholesterol concentration of female rats fed a high-fat high-fructose diet

Parameters	SRC + PDW + PGC	SRCB + FDW + PGC	SRCB + FDW + FGC	SRCB + FDW + GGC	Significance
Glucose (mg/dL)	76.14 ± 6.58^{ab}	65.52 ± 9.23^{a}	76.50 ± 11.86^{b}	66.06 ± 5.37^{ab}	*
Insulin (ng/mL)	42.70 ± 14.03^{a}	49.62 ± 17.62^{a}	47.02 ± 19.45^{a}	44.94 ± 18.40^{a}	ns
HOMA-IR	8.02 ± 2.62^{a}	8.11 ± 3.53^{a}	9.28 ± 5.39^{a}	5.13 ± 2.60^{a}	ns
Liver lipid content (%)	2.64 ± 0.04^{a}	2.40 ± 0.20^{a}	3.20 ± 0.07^{b}	2.90 ± 0.08^{c}	*
Triglyceride (mmol/L)	1.43 ± 0.27^{a}	2.66 ± 0.78^{b}	2.46 ± 0.77^{b}	2.49 ± 0.40^{b}	**
Cholesterol (mmol/L)	1.08 ± 0.16^{a}	1.25 ± 0.06^{a}	1.14 ± 0.14^{a}	1.20 ± 0.10^{a}	ns

n.s. = not significant; * $P < 0.05$; **$P < 0.01$, ^{ab}Within row means with different superscripts are significantly different at $P < 0.05$. The blood glucose concentration of rats to which treatment regimen with fenofibrate was administered was significantly higher ($P < 0.05$) than that of rats to which the control and high-fat high-fructose diet treatment regimen were administered. The triglyceride concentration of rats to which treatment regimens with high-fat high-fructose diet, fenofibrate, and garlic powder was administered was significantly higher ($P < 0.05$) than that of rats fed the control diet. The liver lipid content of the rats fed the high-fat high-fructose diet was significantly lower ($P < 0.05$) compared to that of the rats to which the control diet, the high-fat high-fructose with either fenofibrate or garlic powder as interventions were administered. The liver lipid content of rats fed the high-fat high-fructose diet with either fenofibrate or garlic powder as interventions was significantly higher ($P < 0.05$) than that of rats fed the control diet. The insulin concentration, cholesterol and HOMA-IR indices of the rats were similar ($P > 0.05$) across treatment regimens. SRC + PDW + PGC = standard rat chow + plain drinking water + plain gelatine cube; SRCB + FDW + PGC = standard rat chow with 2%beef tallow (w/w) + 20% fructose in drinking water (w/v) + plain gelatine cube; SRCB + FDW + FGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + fenofibrate gelatine cube (100 mg/kg body mass/day); SRCB + FDW + GGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + garlic powder gelatine cube (100 mg/kg body mass/day); HOMA-IR = homeostatic model of insulin resistance. Data presented as mean ± SD; n =7-10 per treatment.

4.4 Viscera

4.4.1 Liver morphometry

Table 4.5 below shows the effect of garlic powder on the liver mass and NAS of the male rats fed a high-fat high-fructose diet. The male rats fed HFHF diet with fenofibrate as an intervention had significantly heavier ($P < 0.001$) liver masses (absolute and relative to tibia length) compared to that of their counterparts fed the control diet and those fed the high-fat high-fructose diet with garlic powder as an intervention. The rats fed the control diet, the high-fat high-fructose diet and the high-fat high-fructose diet with garlic powder as an intervention had similar ($P > 0.05$) absolute and relative (to body mass and tibia length) liver mass. Ballooning, inflammation, and steatosis of the liver as well as the non-alcoholic fatty liver disease activity score (NAS) of the male rats were similar ($P > 0.05$) across treatment regimens.

Table 4.5: Effect of garlic powder on the liver mass and NAS of male rats fed a high-fat high-fructose diet

Parameters	SRC +PDW + PGC	SRCB +FDW + PGC	SRCB +FDW + FGC	SRCB +FDW + GGC	Significance
Liver (g)	9.87 ± 1.26^{a}	10.93 ± 1.20^{a}	14.38 ± 2.39^{b}	12.18 ± 2.63^{ab}	*
Liver (% TBM)	0.03 ± 0.04^{a}	0.03 ± 0.02^{a}	0.04 ± 0.07^{b}	0.03 ± 0.08^{a}	*
Liver rTL (g/mm)	0.23 ± 0.03^{a}	0.25 ± 0.04^{a}	0.34 ± 0.07^{b}	0.29 ± 0.06^{ab}	***
Ballooning	$0\ (0,0)^{a}$	$0\ (0,0)^{a}$	$0\ (0,0)^{a}$	$0\ (0,0)^{a}$	ns
Inflammation	$2\ (1,2)^{a}$	$2\ (2,3)^{a}$	$2\ (1,2)^{a}$	$2\ (0,2)^{a}$	ns
Steatosis	$0\ (0,0)^{a}$	$0\ (0,1)^{a}$	$0\ (0,0)^{a}$	$0\ (0,0)^{a}$	ns
NAS	$2\ (1,2)^{a}$	$2\ (1,2)^{a}$	$2\ (2,3)^{a}$	$2\ (2,3)^{a}$	ns

ns= not significant, * $P < 0.05$; ***$P < 0.001$. ^{ab}Within rows mean with different superscripts are significantly different at $P < 0.05$. The absolute and relative (to body mass and tibia length) liver mass of the rats to which the control treatment regimen, the high-fat high-fructose-diet and the high-fat high-fructose-diet with garlic powder as an intervention was similar ($P > 0.05$). The livers from rats to which the high-fat high-fructose diet with fenofibrate as an intervention was administered were significantly heavier ($P < 0.05$) compared to the liver mass from their counterparts to which high-fat high-fructose diet with no intervention and or those to which the high-fat high-fructose diet with garlic powder as an intervention was administered. There were no significant differences ($P > 0.05|$) on the liver ballooning, inflammation, steatosis, and non-alcoholic fatty liver disease activity score (NAS) of the rats across treatment regimens. SRC + PDW + PGC = standard rat chow (SRC) + plain drinking water (PDW) + plain gelatine cube (PGC); SRCB + FDW + PGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + plain gelatine cube; SRCB + FDW + FGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + fenofibrate gelatine cube (100 mg/kg body mass/day); SRCB + FDW + GGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + garlic powder gelatine cube (100 mg/kg body mass/day). NAS = non-alcoholic fatty liver disease activity score. Data presented as mean ± SD; n =10 per treatment.

The effects of garlic powder on the liver mass and NAS of the female rats fed a high-fat high-fructose diet are shown in table 4.6 below. The female rats that were fed the high-fat high fructose diet with fenofibrate as an intervention had the heaviest ($P < 0.001$) liver mass compared to the liver mass from rats to which other treatment regimens were administered. The livers of rats to which a high-fat high-fructose diet treatment regimen was administered had significantly higher ($P < 0.05$) inflammation and steatosis compared to the rats to which other treatment regimens were administered. The liver NAS and ballooning of female rats were similar ($P > 0.05$) across treatment regimens.

Table 4.6: Effect of garlic powder on the liver mass and NAS of the female rats fed a high-fat high-fructose diet

Parameters	SRC +PDW + PGC	SRCB +FDW + PGC	SRCB +FDW + FGC	SRCB +FDW + GGC	Significance
Liver (g)	7.56 ± 0.91[a]	7.74 ± 0.54[a]	10.81 ± 1.10[b]	7.57 ± 0.58[a]	***
Liver (%TBM)	0.03 ± 0.03[a]	0.03 ± 0.02[a]	0.04 ± 0.03[b]	0.03± 0.02[a]	***
Liver rTL (g/mm)	0.19± 0.03[a]	0.19 ± 0.02[a]	0.28 ± 0.03[b]	0.19 ± 0.01[a]	***
Ballooning	0 (0,0)[a]	0 (0,0)[a]	0 (0,0)[a]	0 (0,0)[a]	ns
Inflammation	2 (1,2)[a]	3 (2,3)[b]	2 (2,2)[a]	2 (1,2)[a]	**
Steatosis	0 (0,0)[a]	1 (0,1)[b]	0 (0,0)[a]	0 (0,0)[a]	*
NAS	2 (2,2)[a]	2 (2,2)[a]	2 (2,3)[a]	2 (1,3)[a]	ns

ns= not significant, * $P < 0.05$; **$P < 0.01$; ***$P < 0.001$. [ab]Within rows mean with different superscripts are significantly different at $P < 0.05$. The absolute and relative (to body mass and tibia length) liver mass of the female rats to which the high-fat high-fructose diet with fenofibrate as an intervention was administered were significantly heavier ($P < 0.05$) than the liver masses of rats administered with the other treatment regimens. The livers of rats to which the high-fat high-fructose diet treatment regimen was administered had significantly higher ($P < 0.05$) inflammation and steatosis compared to the rats to which other treatment regimens were administered. There were no significant differences in the liver non-alcoholic fatty liver disease activity score (NAS) and ballooning of the female rat across treatment regimens. SRC + PDW + PGC = standard rat chow (SRC) + plain drinking water (PDW) + plain gelatine cube (PGC); SRCB + FDW + PGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + plain gelatine cube; SRCB + FDW + FGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + fenofibrate SRCB + FDW + GGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in gelatine cube (100 mg/kg body mass/day); drinking water (w/v) + garlic powder gelatine cube (100 mg/kg body mass/day). NAS = non-alcoholic fatty liver disease activity score. Data presented as mean ± SD; n =10 per treatment.

Representative liver histology photo sections (H and E staining, 400 X magnification) of male rats from the different treatment groups are shown in Figure 4.11 below.

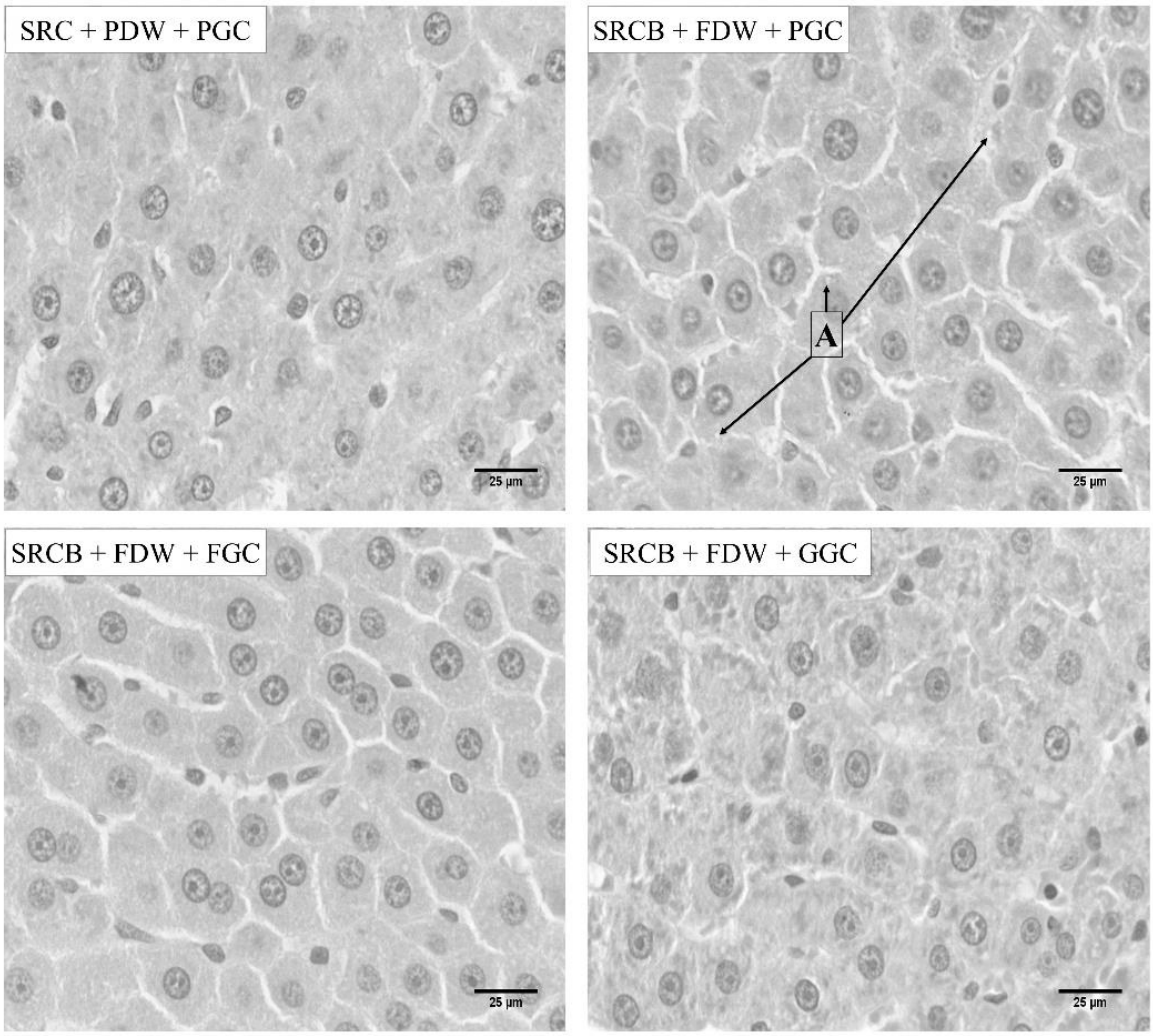


Figure 4.11: Photos of sections showing the liver histology (H and E staining, 400 X magnification) of male rats following the treatment regimens.

Arrows A point to microvesicular steatosis. SRC + PDW + PGC = standard rat chow (SRC) + plain drinking water (PDW) + plain gelatine cube (PGC); SRCB + FDW + PGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + plain gelatine cube; SRCB + FDW + FGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + fenofibrate SRCB + FDW + GGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in gelatine cube (100 mg/kg body mass/day); drinking water (w/v) + garlic powder gelatine cube (100 mg/kg body mass/day).

Microvesicular steatosis was observed in male rats that were fed a high-fat high-fructose diet (Figure 4.11). The microvesicular steatosis was not present in male rats that were fed the HFHF diet with fenofibrate and/or garlic powder (Figure 4.11). Representative liver histology photo sections (H and E staining, 400 X magnification) of female rats from the different treatment groups are shown in Figure 4.12 below.

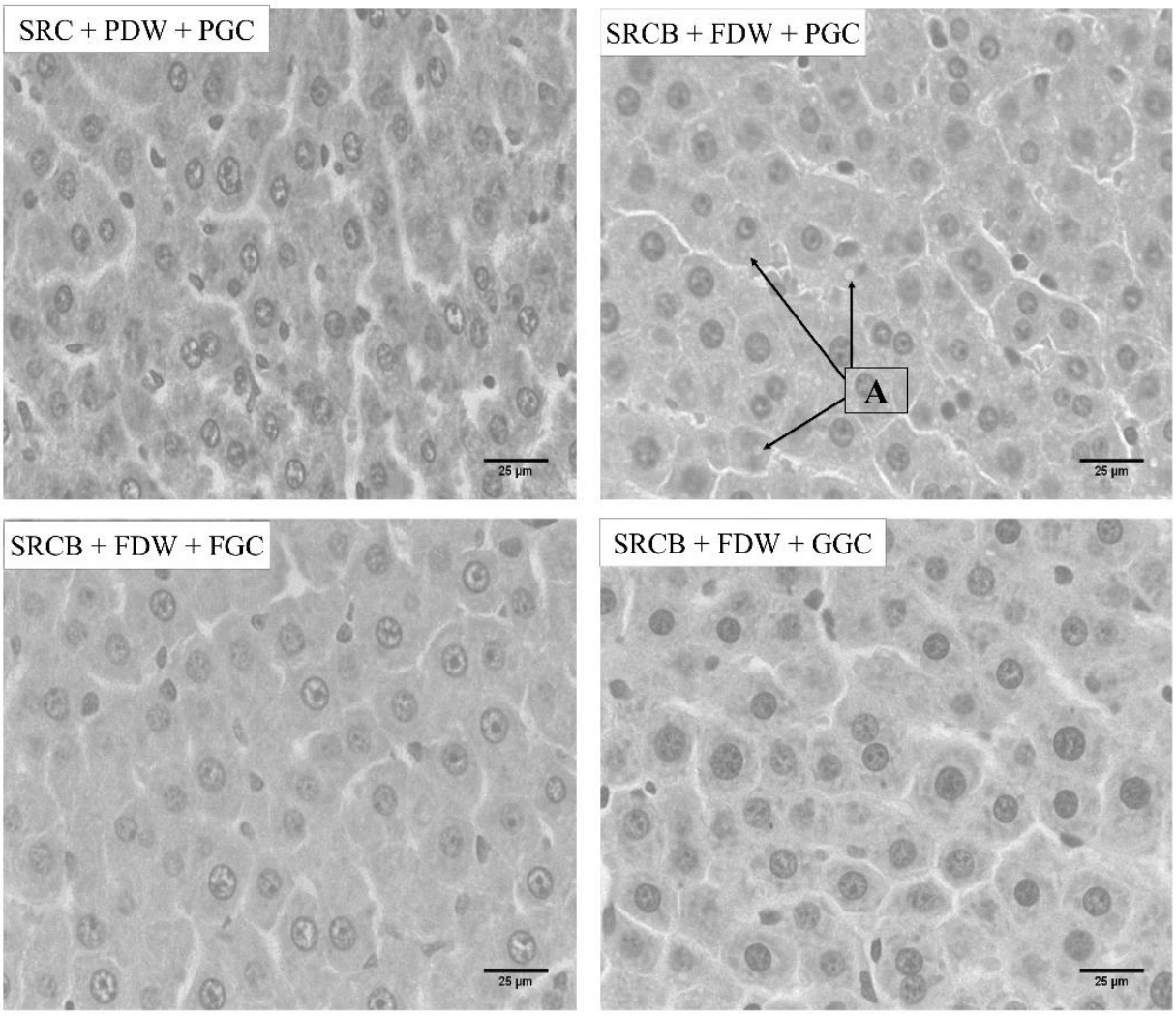


Figure 4.12: Photos of sections showing the liver histology (H and E staining, 400 X magnification) of female rats following the treatment regimens.

Arrows A show microvesicular steatosis. SRC + PDW + PGC = standard rat chow (SRC) + plain drinking water (PDW) + plain gelatine cube (PGC); SRCB + FDW + PGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + plain gelatine cube; SRCB + FDW + FGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + fenofibrate SRCB

+ FDW + GGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in gelatine cube (100 mg/kg body mass/day); drinking water (w/v) + garlic powder gelatine cube (100 mg/kg body mass/day).

Microvesicular steatosis was observed in female rats that were fed a high-fat high-fructose diet (Figure 4.12). The microvesicular steatosis was not present in female rats that were fed the HFHF diet with fenofibrate and/or garlic powder (Figure 4.12).

4.4.2 Kidney morphometry

Table 4.7 below shows the effect of garlic powder on kidney morphometry of male rats fed a high-fat high-fructose diet. The absolute relative (to tibia length) masses of the kidneys were similar ($P > 0.05$) across treatment regimens however relative to body mass kidneys from the rats fed a high-fat high-fructose diet with fenofibrate as an intervention had the heaviest ($P = 0.0017$) kidneys. The kidneys' glomeruli number and density, corpuscular area, glomerular tuft area and urinary space area of rats were similar ($P > 0.05$) across treatment regimens.

Table 4.7: Effect of garlic powder on kidney morphometry of male rats fed a high-fat high-fructose diet

Parameters	SRC + PDW + PGC	SRCB + FDW + PGC	SRCB + FDW + FGC	SRCB + FDW + GGC	Significance
Kidney (g)	2.14 ± 0.29[a]	2.26 ± 0.21[a]	2.45 ± 0.23[a]	2.4 ± 0.30[a]	ns
Kidney (%TBM)	0.61 ± 0.03[a]	0.62 ± 0.04[a]	0.71 ± 0.05[b]	0.67 ± 0.08[a]	**
Kidney rTL (g/mm)	0.05 ± 0.07[a]	0.05 ± 0.04[a]	0.06 ± 0.07[a]	0.06 ± 0.08[a]	ns
Number of glomeruli	7.40 ± 1.60[a]	8.40 ± 2.78[a]	6.86 ± 1.97[a]	7.46 ± 1.3[a]	ns
Glomerular density ($N/\mu m^2 * 10^{-6}$)	5.65 ± 1.22[a]	7.12 ± 2.88[a]	5.24 ± 1.50[a]	5.70 ± 0.99[a]	ns
Corpuscular area (μm^2)	6542 ± 1930[a]	7816 ± 9952[a]	7816 ± 9952[a]	6542 ± 1644[a]	ns
Glomerular tuft area ($N/\mu m^2$)	4317 ± 1283[a]	4513 ± 1621[a]	4513 ± 1621[a]	4397 ± 1235[a]	ns
Urinary space area ($N/\mu m^2$)	2225 ± 647[a]	3303 ± 8334[a]	3303 ± 8334[a]	2145 ± 409[a]	ns

n.s = not significant; **P < 0.01, [ab]Within rows means with different superscripts are significantly different at P < 0.05. The kidney masses (absolute and relative to tibia length) glomeruli number and density, corpuscular area, glomerular tuft area and urinary space area of the rats were similar (P > 0.05) across treatment regimens. Relative to body mass, kidneys from the rats fed a high-fat high-fructose diet with fenofibrate as an intervention were significantly heavier (P < 0.001) compared to kidneys from rats administered other treatment regimens. SRC + PDW + PGC = standard rat chow (SRC) + plain drinking water (PDW) + plain gelatine cube (PGC); SRCB + FDW + PGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + plain gelatine cube; SRCB + FDW + FGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + fenofibrate gelatine cube (100 mg/kg body mass/day); SRCB + FDW + GGC= standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + garlic powder gelatine cube (100 mg/kg body mass/day). Data presented as mean ± SD; n =3-10 per treatment

Table 4.8 below shows the effect of garlic powder on the kidney morphometry of female rats fed a high-fat high-fructose diet. The masses, glomeruli number and density, corpuscular area, glomerular tuft area and urinary space area of the kidneys from rats across treatment regimens were similar ($P > 0.05$).

Table 4.8: Effect of garlic powder on the kidney morphometry of female rats fed a high-fat high-fructose diet

Parameters	SRC +PDW + PGC	SRCB +FDW + PGC	SRCB +FDW + FGC	SRCB +FDW + GGC	Significance
Kidneys (g)	1.59 ± 0.18[a]	1.65 ± 0.12[a]	1.76 ± 0.19[a]	1.62 ± 0.12[a]	ns
Kidneys (%TBM)	0.64 ± 0.04[a]	0.65 ± 0.04[a]	0.68 ± 0.04[a]	0.64 ± 0.04[a]	ns
Kidneys rTL (g/mm)	0.04 ± 0.04[a]	0.04 ± 0.04[a]	0.05 ± 0.05[a]	0.04 ± 0.03[a]	ns
Number of glomeruli	11.33 ± 1.72[a]	7.93 ± 2.91[a]	10.13 ± 1.85[a]	7.60 ± 2.55[a]	ns
Glomeruli density ($N/\mu m^2 * 10^{-6}$)	8.66 ± 1.33[a]	6.06 ± 2.23[a]	7.74 ± 1.42[a]	5.86 ± 1.97[a]	ns
Corpuscular area ($N/\mu m^2$)	6826 ± 9910[a]	5833 ± 1972[a]	5741 ± 1420[a]	5257 ± 1612[a]	ns
Glomerular tuft area ($N/\mu m^2$)	5436 ± 10071[a]	4412 ± 1680[a]	4160 ± 1164[a]	3913 ± 1433[a]	ns
Urinary space area ($N/\mu m^2$)	1390 ± 310.80[a]	1421 ± 292[a]	1581 ± 256[a]	1344 ± 179[a]	ns

ns= not significant, [a]Within rows means with similar superscripts are not significantly different at $P < 0.05$. The kidney masses, glomeruli number and density, corpuscular area, glomerular tuft area and urinary space area of rats to which the four treatment regimens were administered were similar ($P > 0.05$). SRC + PDW + PGC = standard rat chow (SRC) + plain drinking water (PDW) + plain gelatine cube (PGC); SRCB + FDW + PGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + plain gelatine cube; SRCB + FDW + FGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + fenofibrate gelatine cube (100 mg/kg body mass/day); SRCB + FDW + GGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + garlic powder gelatine cube (100 mg/kg body mass/day). Data presented as mean ± SD; n = 3-10 per treatment.

Representative kidney histology photo sections (H and E staining, 400 X magnification) of male rats from the different treatment groups are shown in Figure 4.13 below.

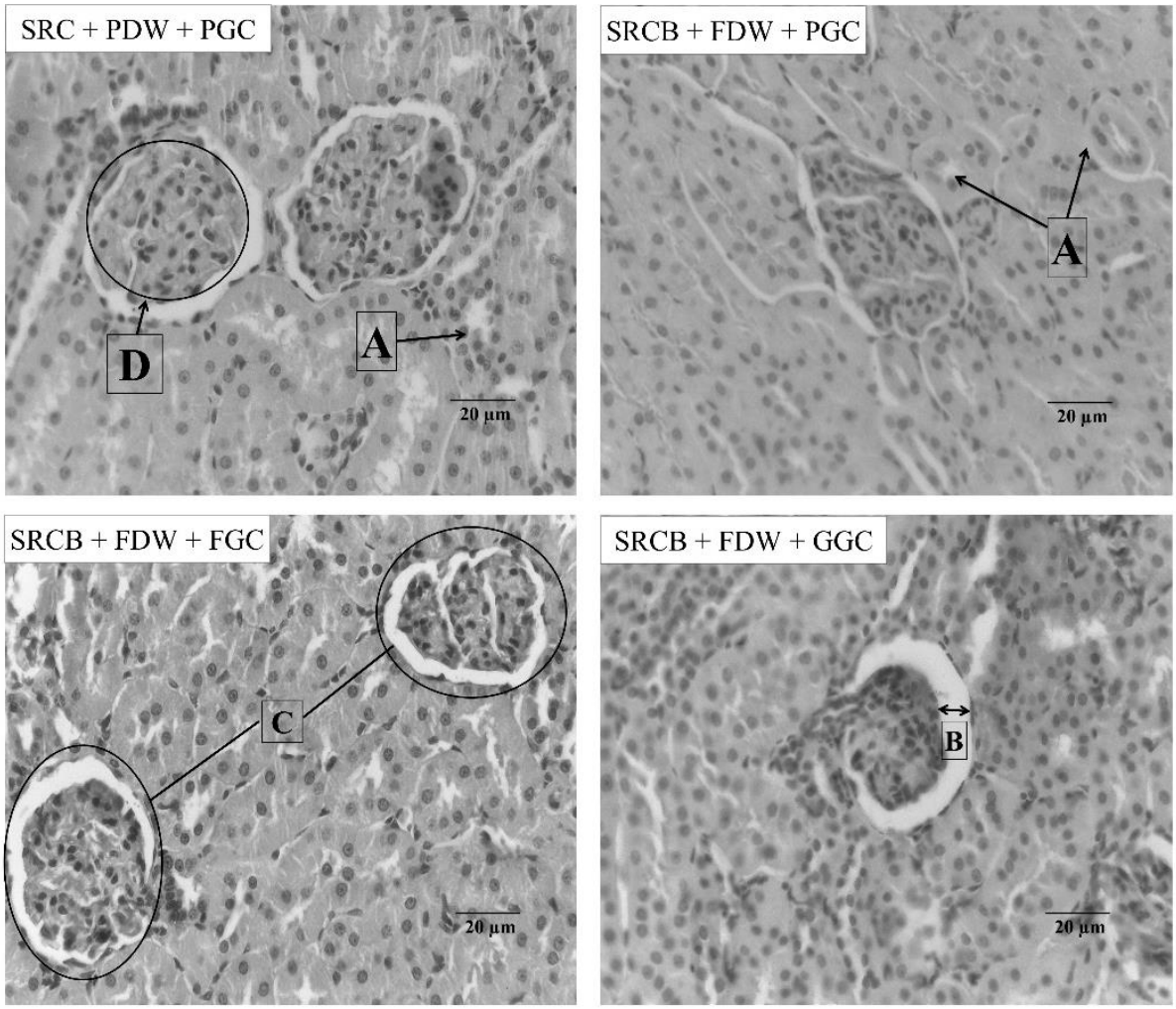


Figure 4.13: Photos of sections showing the kidney histology (H and E staining, 400 X magnification) of male rats following the treatment regimens.

Arrows A point to the renal tubule, arrow B points to urinary space, circles C show the corpuscular tuft area and circle D shows the glomerular tuft area. SRC + PDW + PGC = standard rat chow (SRC) + plain drinking water (PDW) + plain gelatine cube (PGC); SRCB + FDW + PGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + plain gelatine cube; SRCB + FDW + FGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + fenofibrate SRCB + FDW + GGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in gelatine cube (100 mg/kg body mass/day); drinking water (w/v) + garlic powder gelatine cube (100 mg/kg body mass/day).

There were no differences in the kidney histological sections of male rats. Representative kidney histology photo sections (H and E staining, 400 X magnification) of female rats from the different treatment groups are shown in Figure 4.14 below.

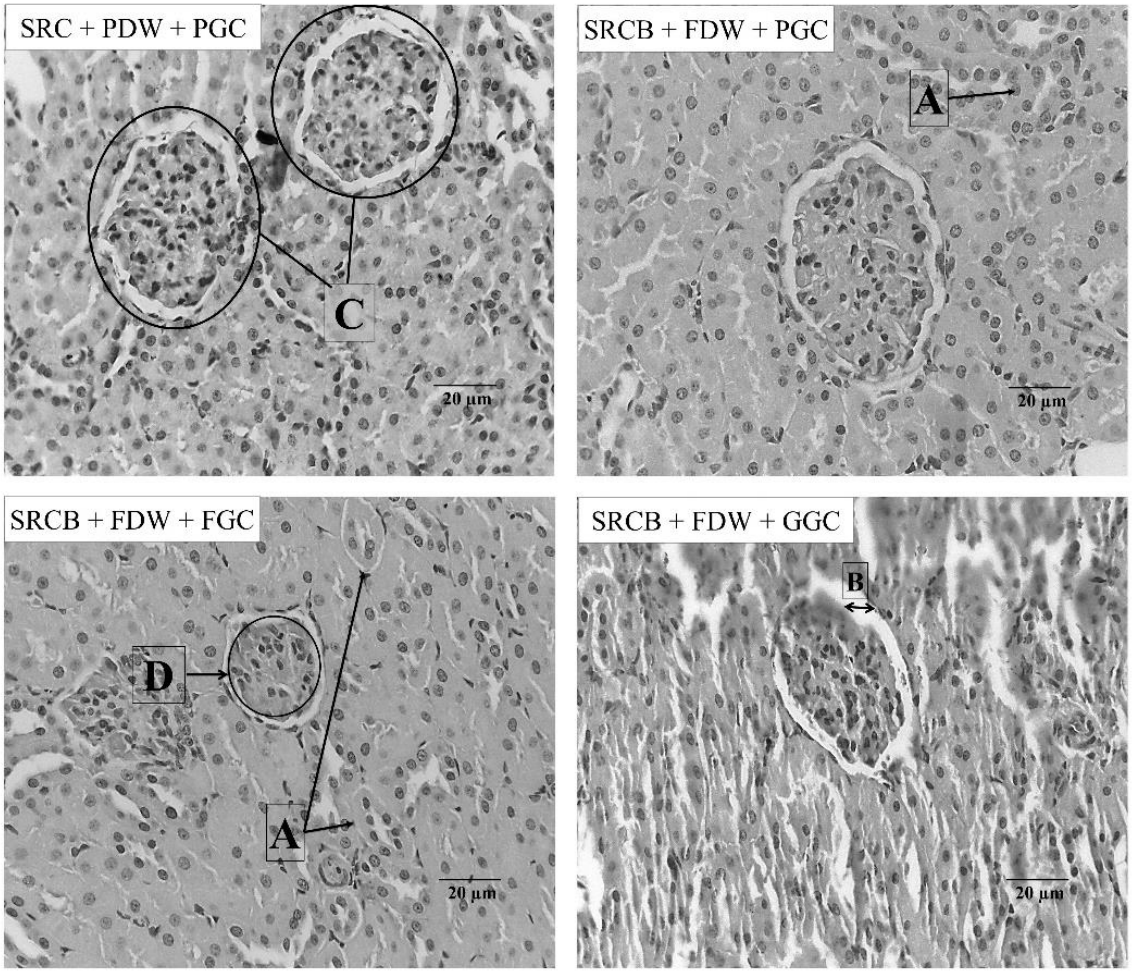


Figure 4.14: Photos of sections showing the kidney histology (H and E staining, 400 X magnification) of female rats following the treatment regimens.

Arrows A point to the renal tubule, arrow B points to urinary space, circles C show the corpuscular tuft area and circle D shows the glomerular tuft area. SRC + PDW + PGC = standard rat chow (SRC) + plain drinking water (PDW) + plain gelatine cube (PGC); SRCB + FDW + PGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + plain gelatine cube; SRCB + FDW + FGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + fenofibrate SRCB + FDW + GGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in gelatine cube (100 mg/kg body mass/day); drinking water (w/v) + garlic powder gelatine cube (100 mg/kg body mass/day).

There were no differences in the kidney histological sections of female rats.

4.4.3 Other viscera

Table 4.9 below shows the effect of garlic powder on the mass and length (where necessary) the small and large intestines, pancreas, visceral and epididymal fat of the male rats fed a high-fat high-fructose diet. The absolute mass of the small intestines (SI) of the male rats fed a high-fat high-fructose diet and that of rats fed a high-fat high-fructose diet with fenofibrate and/or garlic powder as an intervention was significantly higher ($P = 0.0008$) than that from counterparts fed the control diet. There was no significant difference ($P > 0.05$) in the mass (relative to terminal body mass) and length of the small intestine of the rats across treatment regimens. The mass of the small intestine (relative to tibia length) of the rats fed a high-fat high-fructose diet with garlic powder as an intervention was significantly higher ($P < 0.05$) than that of rats fed the control diet but was however similar ($P > 0.05$) to from counterparts fed a high-fat high-fructose diet and those fed a high-fat high-fructose diet with fenofibrate as an intervention. No significant differences ($P > 0.05$) were observed in the mass of the large intestines and pancreata as well as the length of the large intestine ($P > 0.05$) of the rats across treatment regimens. The male rats fed a high-fat high-fructose diet and those fed high-fat high-fructose diet with garlic powder as an intervention had significantly heavier ($P < 0.0001$) visceral and epididymal fat masses compared to that of rats fed the control diet. The mean epididymal fat mass of rats fed a high-fat high-fructose diet was significantly heavier ($P < 0.05$) compared to that of rats fed the control diet.

Table 4.9: Effect of garlic powder on the viscera mass and length of male rats fed a high-fat high-fructose diet

Parameters	SRC + PDW + PGC	SRCB + FDW + PGC	SRCB + FDW + FGC	SRCB + FDW + GGC	Significance
SI (g)	7.40 ± 0.77^{a}	8.34 ± 0.53^{b}	8.23 ± 0.63^{b}	8.69 ± 0.66^{b}	*
SI (%TBM)	0.02 ± 0.03^{a}	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}	0.02 ± 0.02^{a}	ns
SI rTL (g/mm)	0.17 ± 0.02^{a}	0.19 ± 0.01^{ab}	0.19 ± 0.02^{ab}	0.20 ± 0.02^{b}	**
SI (mm)	1321.00 ± 129.7^{a}	1323.00 ± 66.51^{a}	1320.00 ± 50.11^{a}	1331.00 ± 74.75^{a}	ns
LI (g)	1.49 ± 0.17^{a}	1.51 ± 0.16^{a}	1.51 ± 0.17^{a}	1.55 ± 0.21^{a}	ns
LI (%TBM)	0.04 ± 0.06^{a}	0.04 ± 0.05^{a}	0.04 ± 0.05^{a}	0.04 ± 0.03^{a}	ns
LI rTL (g/mm)	0.04 ± 0.03^{a}	0.04 ± 0.04^{a}	0.04 ± 0.04^{a}	0.04 ± 0.06^{a}	ns
LI (mm)	214.00 ± 20.66^{a}	212.00 ± 16.19^{a}	222.00 ± 7.89^{a}	214.00 ± 13.50^{a}	ns
Pancreas (g)	1.23 ± 0.39^{a}	1.53 ± 0.29^{a}	1.43 ± 0.42^{a}	1.64 ± 0.43^{a}	ns
Pancreas (%TBM)	0.03 ± 0.10^{a}	0.04 ± 0.07^{a}	0.04 ± 0.10^{a}	0.04 ± 0.10^{a}	ns
Pancreas rTL (g/mm)	0.03 ± 0.01^{a}	0.04 ± 0.01^{a}	0.03 ± 0.01^{a}	0.04 ± 0.01^{a}	ns
Visceral fat (g)	5.38 ± 2.99^{a}	10.79 ± 2.46^{b}	8.05 ± 1.82^{ab}	10.08 ± 0.88^{b}	*
Visceral fat (%TBM)	0.02 ± 0.06^{a}	0.03 ± 0.06^{b}	0.02 ± 0.06^{ab}	0.03 ± 0.04^{b}	*
Visceral fat rTL (g/mm)	0.13 ± 0.07^{a}	0.25 ± 0.06^{b}	0.19 ± 0.05^{ab}	0.23 ± 0.03^{b}	***
Epididymal fat (g)	2.28 ± 0.58^{a}	3.05 ± 0.67^{b}	2.36 ± 0.60^{ab}	2.69 ± 0.51^{ab}	*
Epididymal fat (%TBM)	0.06 ± 0.01^{a}	0.08 ± 0.01^{b}	0.07 ± 0.01^{ab}	0.07 ± 0.01^{ab}	*
Epididymal fat rTL (g/mm)	0.05 ± 0.01^{a}	0.07 ± 0.02^{b}	0.06 ± 0.02^{a}	0.06 ± 0.01^{a}	*

n.s = not significant. * $P < 0.05$; **$P < 0.01$; ***$P < 0.0001$, ^{ab}Within rows mean with different superscripts are significantly different at $P < 0.05$. The small intestine mass (relative to body mass) and length of male rats were similar ($P > 0.05$) across treatment regimens. The absolute mass of the small intestines of rats fed the high-fat high-fructose diet and those fed the high-fat high-fructose diet with fenofibrate and/or garlic powder as an intervention was significantly higher ($P < 0.05$) compared to that of counterpart fed the control diet. The small intestine mass (absolute and

relative to tibia length) of rats fed the high-fat high-fructose diet with garlic powder as an intervention was significantly higher ($P < 0.05$) compared to that from rats fed the control diet, but similar ($P > 0.05$) to that of rats fed the high-fat high-fructose diet and a high-fat high-fructose diet with fenofibrate as an intervention. No significant differences ($P> 0.05$) were observed in the mass and length of the large intestine and mass of the pancreata ($P > 0.05$) of the rats across treatment regimens. The visceral fat and epididymal fat masses of the rats fed the high-fat high-fructose diet were significantly heavier ($P < 0.05$) compared to that from counterparts fed the control diet. The visceral fat mass of rats fed the high-fat high-fructose diet with fenofibrate as an intervention was similar ($P > 0.05$) to that of counterparts fed the control diet. The epididymal fat mass of rats fed the high-fat high-fructose diet with either fenofibrate and or garlic as an intervention was similar ($P > 0.05$) to the epididymal fat mass of rats fed the control diet. The large intestine's absolute mass, mass relative to terminal body mass, LI length and mass relative to tibia length of rats to which the four treatment regimens were administered were not significantly different ($P > 0.05$). SI = small intestine; LI = large intestine; %TBM = relative to terminal body mass; rTL = relative to tibia length. SRC + PDW + PGC = standard rat chow (SRC) + plain drinking water (PDW) + plain gelatine cube (PGC); SRCB + FDW + PGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + plain gelatine cubes; SRCB + FDW + FGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + fenofibrate gelatine cube (100 mg/kg body mass/day); SRCB + FDW + GGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + garlic powder gelatine cube (100 mg/kg body mass/day). Data presented as mean ± SD; n =10 per treatment.

Table 4.10 below shows the effect of garlic powder on the mass and length (where necessary) of the small and large intestines, pancreata, visceral fat of the female rats fed a high-fat high-fructose diet. Across treatment regimens, the mass and length of the small and large intestines, the mean masses of the pancreata and visceral fat were similar ($P > 0.05$) across treatment regimens. Rats fed a high-fat high-fructose diet had significantly ($P = 0.0314$) lighter large intestine (relative to body) compared to that from rats fed the control diet. The rats fed a high-fat high-fructose diet had significantly heavier ($P = 0.0274$) mean visceral fat (relative to body mass) masses compared to that from rats fed other treatment regimens.

Table 4.10: Effect of garlic powder on the viscera's mass and length of female rats fed a high-fat high-fructose diet

Parameters	SRC + PDW + PGC	SRCB + FDW + PGC	SRCB + FDW + FGC	SRCB + FDW + GGC	Significance
SI (g)	5.81 ± 1.88[a]	6.42 ± 0.43[a]	6.89 ± 0.63[a]	6.56 ± 0.46[a]	ns
SI (%TBM)	0.02 ± 0.08[a]	0.03 ± 0.02[a]	0.03 ± 0.01[a]	0.03 ± 0.01[a]	ns
SI rTL (g/mm)	0.15 ± 0.05[a]	0.16 ± 0.01[a]	0.18 ± 0.02[a]	0.16 ± 0.01[a]	ns
SI (mm)	1165 ± 128[a]	1190 ± 108[a]	1118 ± 295.70[a]	1130 ± 154.40[a]	ns
LI (g)	1.39 ± 0.12[a]	1.20 ± 0.15[a]	1.31 ± 0.26[a]	1.25 ± 0.15[a]	ns
LI (%TBM)	0.06 ± 0.08[a]	0.05 ± 0.05[b]	0.05 ± 0.07[a]	0.05 ±0.06[a]	*
LI rTL (g/mm)	0.04 ± 0.03[a]	0.03 ± 0.04[a]	0.03 ± 0.07[a]	0.03 ± 0.03[a]	ns
LI (mm)	210 ± 17.64[a]	195 ± 19.00[a]	192 ± 17.98[a]	201 ± 15.06[a]	ns
Pancreas (g)	1.09 ± 0.24[a]	1.05 ± 0.30[a]	1.23 ± 0.20[a]	1.15 ± 0.31[a]	ns
Pancreas (%TBM)	0.04 ± 0.10[a]	0.04 ± 0.12[a]	0.04 ± 0.08[a]	0.04 ± 0.12[a]	ns
Pancreas rTL	0.03 ± 0.06[a]	0.03 ± 0.07[a]	0.03 ± 0.05[a]	0.03 ± 0.08[a]	ns
Visceral fat (g)	9.15 ± 3.45[a]	11.80 ± 2.85[a]	11.26 ± 2.38[a]	11.82 ± 1.64[a]	ns
Visceral fat (%TBM)	0.04 ± 0.01[a]	0.05 ± 0.01[b]	0.04 ± 0.07[a]	0.05 ± 0.06[a]	*
Visceral fat rTL (g/mm)	0.23 ± 0.09[a]	0.30 ± 0.08[a]	0.29 ± 0.06[a]	0.29 ± 0.04[a]	ns

n.s = not significant. * $P < 0.05$. [ab]Within rows mean with different superscripts are significantly different at $P < 0.05$. There were no significant differences ($P > 0.05$) in the mass and length of the small intestine mass, mass of the pancreata and visceral fat mass of the rats across treatment regimens. Relative to body mass the mass of the large intestine and visceral fat of the rats fed the high-fat high-fructose diet were significantly higher ($P < 0.05$) compared to that of their counterparts administered the other treatment regimens. SI = small intestine; LI = large intestine; %TBM = relative to terminal body mass; rTL = relative to tibia length. SRC + PDW + PGC = standard rat chow (SRC) + plain drinking water (PDW) + plain gelatine cube (PGC); SRCB + FDW + PGC = standard rat chow with

2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + plain gelatine cube; SRCB + FDW + FGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + fenofibrate gelatine cube (100 mg/kg body mass/day); SRCB + FDW + GGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + garlic powder gelatine cube (100 mg/kg body mass/day). Data presented as mean ± SD; n =10 per treatment.

4.5 Surrogate markers of hepatic and renal health

The effect of garlic powder on the plasma creatinine and blood urea nitrogen concentration, BUN-creatinine ratio, ALT and ALKP activities of the male rats fed a high-fat high-fructose diet are shown in table 4.11 below. The blood urea nitrogen concentration of rats fed a high-fat high-fructose diet was significantly lower ($P = 0.0116$) than that of their counterparts fed other treatment regimens. There were no significant differences ($P > 0.05$) in the plasma creatinine concentration as well as the ALT and ALKP activities of the rats across treatment regimens. The urea: creatinine ratio of rats fed a high-fat high-fructose diet with fenofibrate as an intervention was significantly higher ($P = 0.0123$) compared to that of rats fed a high-fat high-fructose diet but similar ($P > 0.05$) to that of rats fed either the control or the high-fat high-fructose diet with garlic powder as an intervention.

Table 4.11: Effect of garlic powder on plasma creatinine, blood urea nitrogen concentration, BUN-creatinine ratio, ALT and ALKP activities of male rats fed a high-fat high-fructose diet

Parameters	SRC + PDW + PGC	SRCB + FDW + PGC	SRCB + FDW + FGC	SRCB + FDW + GGC	Significance
Creatinine (µmol/L)	27.57 ± 3.36[a]	20.29 ± 14.24[a]	24.29 ± 3.25[a]	13.29 ± 17.06[a]	ns
BUN (mmol/L)	5.11 ± 1.10[ab]	4.46 ± 0.62[b]	6.26 ± 0.84[a]	5.30 ± 1.04[ab]	**
ALT (U/L)	66.14 ± 15.64[a]	55.14 ± 8.61[a]	55.43 ± 15.09[a]	68.00 ± 16.94[a]	ns
ALKP (U/L)	138.00 ± 23.78[a]	163.70 ± 42.60[a]	182.30 ± 16.47[a]	172.90 ± 43.87[a]	ns
BUN: Creatinine ratio	51.2 ± 9.60[ab]	24 ± 22.79[a]	67 ± 14.98[b]	29.4 ± 27.32[ab]	*

n.s = not significant. * P < 0.05, ** P < 0.01. [abc]Within rows mean with different superscripts are significantly different at P < 0.05. The creatinine concentration, the ALT and ALKP activities of the male rats were similar (P > 0.05) across treatment regimens. The blood urea nitrogen concentration of rats fed the high-fat high-fructose diet was significantly lower (P = 0.0116) compared to that of their counterparts fed the control diet and those fed the high-fat high-fructose diet with fenofibrate as an intervention. The BUN: creatinine ratio of rats fed the high-fat high-fructose diet with fenofibrate as an intervention was significantly higher (P = 0.0123) than that of rats fed a high-fat high-fructose diet but similar (P > 0.05) to the rats fed the control diet and that of rats fed high-fat high-fructose diet with garlic powder as an intervention. BUN = blood urea nitrogen, ALKP = alkaline phosphatase, ALT = alanine aminotransferase. SRC + PDW + PGC = standard rat chow (SRC) + plain drinking water (PDW) + plain gelatine cube (PGC); SRCB + FDW + PGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + plain gelatine cube; SRCB + FDW + FGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + fenofibrate gelatine cube (100 mg/kg body mass/day); SRCB + FDW + GGC = standard rat chow with 2% beef tallow(w/w) + 20% fructose in drinking water (w/v) + garlic powder gelatine cube (100 mg/kg body mass/day). Data presented as mean ± SD; n =7 per treatment.

The effect of garlic powder on the plasma creatinine and blood urea nitrogen concentration, BUN-creatinine ratio and plasma ALT and ALKP activities of the female rats fed a high-fat high-fructose diet are shown in table 4.12 below. There were no significant differences ($P > 0.05$) in the plasma creatinine, blood urea nitrogen concentration, BUN: creatinine ratio as well as the plasma ALKP and ALT activities of the rats across treatment regimens.

Table 4.12: Effect of garlic powder on plasma creatinine, blood urea nitrogen concentration, BUN-creatinine ratio, ALT and ALKP activities of female rats fed a high-fat high-fructose diet

Parameters	SRC +PDW + PGC	SRCB + FDW + PGC	SRCB + FDW + FGC	SRCB + FDW + GGC	Significance
Creatinine (μmol/L)	32.14 ± 6.34[a]	25.71 ± 12.53[a]	26.71 ± 4.03[a]	28.43 ±3.16[a]	ns
BUN (mmol/L)	4.83 ± 1.01[a]	3.84 ± 0.65[a]	4.10 ± 1.10[a]	4.19 ± 0.68[a]	ns
ALT (U/L)	45.71 ± 7.10[a]	46.14 ± 8.51[a]	54.43 ± 14.46[a]	41.57 ± 4.35[a]	ns
ALKP (U/L)	119.4 ± 21.43[a]	115.9 ±11.63[a]	172.6 ± 80.31[a]	118.3 ± 23.01[a]	ns
BUN: Creatinine ratio	27.31 ± 5.33[a]	21.87 ± 11.88[a]	22.61 ± 2.93[a]	24.24 ± 2.48[a]	ns

n.s = not significant. [a]Within rows means with similar superscripts are not significantly different at P > 0.05. The plasma creatinine, blood urea nitrogen concentration, BUN-creatinine ratio, ALT and ALKP activities of the rats were similar (P > 0.05) across treatment regimens. BUN = blood urea nitrogen, ALKP = alkaline phosphatase, ALT = alanine aminotransferase. SRC + PDW + PGC = standard rat chow (SRC) + plain drinking water (PDW) + plain gelatine cube (PGC); SRCB + FDW + PGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + plain gelatine cube; SRCB + FDW + FGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + fenofibrate gelatine cube (100 mg/kg body mass/day); SRCB + FDW + GGC = standard rat chow with 2% beef tallow (w/w) + 20% fructose in drinking water (w/v) + garlic powder gelatine cube (100 mg/kg body mass/day). Data presented as mean ± SD; n =7 per treatment.

CHAPTER 5: DISCUSSION

The chapter discusses findings of the effects of garlic powder on the growth performance, haematological parameters, circulating and stored metabolites, GIT and other viscera and the general health profile of growing male and female Sprague Dawley rats fed a high-fat high-fructose diet without and with fenofibrate and/or garlic powder as an intervention. The chapter gives meaning to the results of the study and draws attention to the possible application of the findings by comparing the results with the findings of other relevant studies.

5.0 Phenolic content

In the current study, the garlic powder contained 0.082mgGAE/g total phenolics. Nuutila *et al.* (2003) reported total phenolic content of 0.075-0.080mgGAE/g) in garlic. The total phenolics content reported for the garlic powder used in the current study falls within the range reported by Nuutila *et al.* (2003). Phenolic compounds are associated with the health beneficial properties of garlic (Yin and Cheng, 1998).

5.1 Growth performance

5.1.1 Body mass

In the current study feeding growing male and female Sprague Dawley rats with a high-fat high-fructose diet without and/or with either fenofibrate or garlic powder as an intervention was observed to have had no effect on terminal body mass of the rats compared to rats fed the control diet (Figures 4.1 and 4.2). In adult rats feeding a high-fat or a high-fructose diet and/or a combination of both have been observed to result in increased body mass (Jurgens *et al.*, 2005; Panchal *et al.*, 2011). This was not the case in the current study. Despite the extra calories in the form of fructose and fat given to the rats, their failure to respond with a significant increase in body mass could have been due to the fact that since they were growing animals, most of the "extra calories" (energy) could have been channelled towards growth (Ghezzi *et al.*, 2012). Importantly, the similarity in the body mass of the rats to which fenofibrate and/or garlic powder were administered as an intervention to the body mass of the rats fed the control diet and that of rats fed the high-fat high-fructose diet suggests that fenofibrate and garlic powder had no adverse effects on the growth performance of the rats. Research shows that the consumption of high-

fructose and high-fat diet promote an increase in body mass in adult rats (Lozano *et al.*, 2016), but it has to be noted that in the current study the rats had access to the fructose solution from the weanling growth stage to adulthood. The differences in the response to the consumption of a fructose solution seem to be age-dependent with adult animals, including human beings, accreting more fat, hence body mass, on consumption of extra calories (Panchal and Brown, 2011; Mamikutty *et al.*, 2014). Jurgens *et al.* (2005) confirm the association between an increase in body mass and the consumption of fructose in adult rats. While garlic has been used as a growth promoter in poultry and pig production (Chen *et al.*, 2008; Toghyani *et al.*, 2011), results of the current study suggest that garlic powder did not exert any growth promoting effect on the rats. Ali *et al.* (2000) reported that intervening with garlic in rats fed a high-cholesterol high-fructose had no significant effect on total body weight of female SD rats over 6 weeks which finding is mirrored by results of the current study. Garlic has been reported to inhibit cholesterol absorption from the GIT and to stimulate the secretion of adrenalin (Djankpa *et al.*, 2012). Through these two physiological effects, garlic reduces the channelling of cholesterol and probably other nutrients into the body for fat accretion while adrenalin mediates increased metabolism (Djankpa *et al.*, 2012); all of which would cause an increase in body mass.

5.1.2 Linear growth

The use of body mass and its associated derivatives is confounded by the fact that body mass is influenced by the hydration state, gastrointestinal tract (GIT) content and visceral fat size (Owens *et al.*, 1995) resulting in it (body mass) being an inaccurate measure of growth performance. Long bones, such as tibiae and femora respond to growth hormone in a dose-dependent manner (Eshet *et al.*, 2004; Venken *et al.*, 2008), making the use of long bone indices a more accurate measure of growth performance (Panchal *et al.*, 2011; Mamikutty *et al.*, 2014). In the current study feeding rats a high-fat high-fructose diet and/or a high-fat high-fructose diet with either fenofibrate or garlic powder as an intervention did not affect the rats' tibiae and femora masses, lengths and Seedor ratios, when compared to those of the rats fed the control diet (Tables 4.1 and 4.2). These findings suggest that the high-fat high-fructose diet, as well as the fenofibrate and/or the garlic powder, did not compromise the growth performance of the rats as measured by these stated bone parameters. While the consumption of a high-fat diet was reported

to negatively affect long bone mass in rats (Lac *et al.*, 2008), Tsanzi *et al.* (2008) observed that the consumption of fructose had no effect on bone indices in rats. The results of the current study are therefore in agreement with the findings of Tsanzi *et al.* (2008) but are in disagreement with the observations of Lac *et al.* (2008). In the study by Lac *et al.* (2008), a 7.7% of fat was used which had adverse effect on linear growth of rats. A lower percentage (2%) of fat compared to that used in the study by Lac *et al.* (2008) was utilized in the current study, this could be a possible explanation why there were no adverse effects on the rats' linear growth in this study.

5.2 Haematological parameters

5.2.1 Packed cell volume

In this present study, the consumption of a high-fat high-fructose diet and/or the high-fat high-fructose diet with either fenofibrate or garlic powder as an intervention had no significant effect on the packed cell volume of female rats (Figure 4.4). However, in male rats, the consumption of the high-fat high-fructose diet and/or the high-fat high-fructose diet with either fenofibrate or garlic powder as an intervention resulted in significant lower PCV compared to that of their counterparts fed the control diet (Figure 4.3). In the female rats, these findings suggest that the high-fat high-fructose diet did not compromise the rat's PCV and importantly also that both the fenofibrate and garlic powder had no negative effect on the rats' haematocrit. With regards to male rats, these current study findings suggest that the high-fat high-fructose diet has a detrimental effect on their haematocrit as shown by its (PCV) being lowered compared to that of rats fed the control diet. Importantly, this high-fat high-fructose diet-mediated lowering of the male rats' haematocrit was not attenuated by either fenofibrate or garlic powder. It can be inferred from these findings that the high-fat high-fructose diet affects the haematocrit of growing Sprague Dawley rats in a sexually dimorphic manner. In a human study by Heseltine *et al.* (1990), a high-carbohydrate and high-fat diet was shown to decrease packed cell volume.

5.2.2 Erythrocyte osmotic fragility

The erythrocyte cholesterol and fatty acid cell membrane composition are known to be influenced by diet (Kempaiah and Srinivasan, 2002). Cholesterol and fatty acid content of the

cell membrane affects membrane fluidity (Kempaiah and Srinivasan, 2002), with a high cholesterol content known to be associated with the appearance of prominent abnormal spicules on the cell surface, an increase in the internal viscosity of the lipid phases of the membrane, and the eventual decrease in the viability of the erythrocytes [after 8-16 weeks on high-fat diet (Kroes and Ostwald, 1971)] and high fatty acid content having been observed to result in an increase in erythrocyte sensitivity (Ehrström *et al.*, 1981). In the current study, the 50% and 100% haemolysis occurred between 0.3% and 0.5% concentration of the phosphate buffered saline solution and there were no significant differences across treatment regimens (Figures 4.5 and 4.6). The similarity in the osmotic fragility of erythrocytes from the rats in the current study suggests that the high-fat high-fructose diet did not compromise erythrocyte membranes. It can be speculated to mean that this high-calorie diet might not have caused a change in the membrane constituent composition. Importantly, it can be inferred that both fenofibrate and garlic powder when given as interventions in high-fat high-fructose diet fed rats have neither negative nor beneficial effect specifically on erythrocyte membrane fragility. Kempaiah and Srinivasan (2002) reported that the consumption of a high-fat diet alters the lipid profile of erythrocytes cell membranes while the administration of garlic powder was shown to protect the structural integrity of the rats' erythrocyte cell membranes. Erythrocyte membranes of hypercholesterolaemic rats fed a high-fat diet were observed to be fragile and this fragility was attenuated by garlic powder (Kempaiah and Srinivasan, 2002). In the current study, it can be inferred that the high-fat high-fructose diet did not compromise the structural integrity of the rats' erythrocytes. In their study Kempaiah and Srinivasan (2002) used cholesterol as the dietary fat while in the current study beef tallow, which likely contains several constituencies of fat was used. It can therefore be speculated that the dietary beef tallow, unlike dietary cholesterol, did not compromise erythrocyte membrane fragility.

5.2.3 Oral glucose tolerance test

Elevated fasting basal glucose concentration and/or insufficient or dampened response to insulin are characteristics of impaired glucose handling (American Diabetes Association, 2013). In this study, there were no adverse effects observed on glucose handling by both male and female rats

following a challenge by an oral glucose load (Figures 4.7 and 4.8). It was interesting to note that the time taken to return to the basal glucose concentration following the glucose challenge was similar across treatment regimens. These results suggest that the high-fat high-fructose diet, the fenofibrate and/or garlic powder as interventions did not compromise factors that regulate glucose homeostasis, for example, insulin secretion and sensitivity. These results are in contrast to the observed and reported impaired tolerance to a glucose load by 3-week old male rats that were fed a 20% fructose solution for 6 weeks (Dupas *et al.*, 2016). In the current study the rats were fed a high-fructose high-fat diet unlike in the Dupas et al (2016) where a high-fructose diet was fed. A high-fat diet has been shown to reduce receptors responsible for carbohydrate absorption in the small intestine (Brown *et al.*, 2011). In the current study, it can be speculated that the high dietary fat suppressed carbohydrate absorption, hence the differences in the findings of the current study and others. It has been reported that regular dietary supplementation with garlic helps reduce blood glucose concentration (Drobiova *et al.*, 2011). In the current study, the similarities in the response to an oral glucose challenge by the rats across treatment regimens agrees with the assertion by Drobiova *et al.* (2011) since the glucose handling of rats fed with garlic was similar to the control. Liu *et al.* (2011) reported that fenofibrate causes oxidative stress and inflammation to the pancreas thus results in reduced levels of insulin secretion and diminished ability to handle glucose. The results of this study are at variance with this because there was no hyperglycaemia indicating that insulin secretion and cell sensitivity to insulin were not compromised. The fenofibrate used in a study by Liu *et al.* (2011) was gavaged for 12 weeks to monosodium glutamate induced obese rats. The reason why the findings in the current study differ with that of Liu *et al.* (2011) could be the method and period of administering fenofibrate to the rats and the model with which metabolic derangements were induced.

While the area under the curve following an oral glucose challenge test of the female rats across treatment regimens was similar, that for the male rats fed the high-fat high-fructose diet with either fenofibrate or garlic powder as an intervention were significantly higher compared to that from rats fed the control diet suggesting that both fenofibrate and garlic powder somewhat compromised glucose handling (Figures 4.9 and 4.10). Suwannaphet *et al.* (2010) reported that an increase in AUC in Sprague-Dawley rats fed a high-fructose diet for 8 weeks is indicative of an impaired ability of insulin to stimulate glucose disposal in peripheral tissues. In the current

study, the similarity in the AUC of female rats fed a high-fat high-fructose diet suggests that the insulin-mediated regulation of blood glucose disposal to tissues was not impaired.

Perpetual treatment with fenofibrate leads to the disruption of beta cell function and an impairment in the secretion of glucose-stimulated insulin, thus worsens glucose handling ability (Liu *et al.*, 2011). The findings by Liu *et al.* (2011) are in tandem with the observed effect of fenofibrate in the current study.

5.3 Metabolic substrates and NAFLD parameters

Lozano *et al.* (2016) reported that feeding a high-fructose high-fat diet to adult rats for an 8-month period resulted in increased blood glucose concentration. In the current study in both male and female rats, the feeding of a high-fat high-fructose diet and or the high-fat high-fructose diet with either fenofibrate or garlic as an intervention resulted in similar plasma insulin and cholesterol concentration and HOMA-IR index (Tables 4.3 and 4.4, respectively). These results suggest that the high-fat high-fructose diet did not alter the metabolism of cholesterol, insulin secretion and sensitivity to the secreted insulin. Additionally, results of the current study suggest that both fenofibrate and garlic powder did not affect the metabolism of cholesterol, insulin secretion, and sensitivity. However, in female rats, the consumption of the high-fat high-fructose diet with fenofibrate as an intervention resulted in significantly higher blood glucose concentration (Table 4.4). This suggests that in female rats fenofibrate negatively interferes with mechanisms that regulate glucose homeostasis. The use of either fenofibrate and or GP might cause derangement of blood glucose homeostasis in growing male children since they compromised post-prandial glycaemic control in growing male rats fed an obesogenic diet. Le *et al.* (2009) reported that the consumption of diets rich in fructose caused increased lipid synthesis and its storage in the liver. Other researchers have observed that the consumption of high-fructose diets mediates altered lipid metabolism that manifests as increased plasma triglycerides from increased *de novo* lipid hepatic secretion (Kelley *et al.*, 2004; Faeh *et al.*, 2005).

In the current study, the male rats fed the high-fat high-fructose diet had lower hepatic lipid content and higher blood triglyceride concentration compared to that of rats fed the control diet (Table 4.3). The high-fat high-fructose diet had no effect on steatosis, inflammation, ballooning

and total NAS scores of male rats (Table 4.5). These findings suggest that the high-fat high-fructose diet caused hypertriglyceridaemia but not NAFLD in the male rats. In male rats, results from the current study are at variance with the observation by Le *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 male rats by shuttling triglycerides into circulation. Nonetheless, this atypical finding requires further investigation. Interestingly, the male rats that were fed the high-fat high-fructose diet with either fenofibrate or garlic powder as an intervention had the highest liver lipid content in the present study (Table 4.3). The increase in liver lipid content was accompanied by hypertriglyceridaemia but it did not result in steatosis, inflammation or ballooning (Tables 4.3 and 4.5). These contradictory findings are difficult to explain, however, long-term PPAR-α receptor activation has been shown to upregulate sterol regulatory element-binding protein 1c (SREBP-1c) that stimulate lipogenesis (Yan *et al.*, 2014). Fenofibrate, as well as the phytochemical constituents of garlic, are known to activate PPAR-α receptor (Ortuño Sahagún *et al.*, 2012; Yan *et al.*, 2014). Thus we speculate that the fenofibrate and garlic powder increased liver lipid through the long-term activation of PPAR-α receptor in the present study. The formation (vacuolation) of hepatic steatosis is determined by the expression of stearoyl-CoA desaturase-1 (SCD-1), a lipid-partitioning enzyme (Li *et al.*, 2009). It is possible to have increased total liver lipid content without the development of hepatic steatosis if the expression of SCD-1 enzyme is not upregulated. It can be speculated that in the current study, fenofibrate and garlic powder caused lipogenesis but did not upregulate hepatic SCD-1 expression. The triglyceride concentration of the male rats fed the high-fat high-fructose diet was similar to that of their counterparts fed the high-fat high-fructose diet with either fenofibrate or garlic powder as an intervention (Table 4.3). This suggests that the interventions, fenofibrate and garlic powder, did not protect the male rats against a high-fat high-fructose diet-induced hypertriglyceridaemia. Importantly, current results suggest that both fenofibrate and garlic powder, when used as potential prophylactic agents in male rats fed a high-fat high-fructose diet mediate an increase in hepatic lipid accretion which might lead to hepatic steatosis. Caution, therefore, needs to be taken in the use of both fenofibrate and garlic powder in the prevention of diet-induced (high-fat high-fructose) metabolic derangements in males as they may cause an abnormal deposition of fat in the liver.

Results of the current study show that in female rats consumption of the high-fat high-fructose diet had no effect on the liver lipid content (Table 4.4). However, the high-fat high-fructose diet caused steatosis, inflammation, and hypertriglyceridaemia (Tables 4.4 and 4.6). The findings on liver lipid content are in variance with previous studies that have reported that the consumption of a high-fat high-fructose diet causes increased liver lipid accretion (Lozano *et al.*, 2016). A possible explanation of this finding could be that there was increased shuttling of triglycerides into circulation thus resulting in the observed hypertriglyceridaemia. The presence of steatosis (histological) could be as a result of the upregulation in SCD-1 enzymes that partition lipids in the liver and cause steatosis (Yan *et al.*, 2014). Additionally, inflammation has been implicated in the manifestation of hepatic steatosis (Gao and Tsukamoto, 2016). Thus it could be speculated that the inflammation attributed to the manifestation of hepatic steatosis in high-fat high-fructose diet-fed female rats. The fenofibrate and garlic powder prevented high-fat high-fructose diet-induced steatosis and inflammation but not hypertriglyceridaemia in female rats (Tables 4.4 and 4.6). It could be speculated that fenofibrate and garlic powder prevented the hepatic steatosis by suppressing hepatic SCD-1 enzyme expression and inflammation. Fenofibrate and garlic have been reported to have anti-inflammatory properties (Djankpa *et al.*, 2012; Prasad *et al.*, 2018). Remarkably, the administration of fenofibrate and garlic powder resulted in increased liver lipid accretion (without steatosis) in high-fat high-fructose diet-fed female rats. We speculate that a possible explanation for these findings could be due to long-term PPAR-α activation without an upregulation in the expression of SCD-1 enzymes.

The plasma triglyceride concentration of the female rats fed the high-fat high-fructose diet was similar to that of rats fed the high-fat high-fructose diet with either fenofibrate or garlic powder as an intervention (Table 4.4). These findings suggest that in female rats, the high-fat high-fructose diet-induced hypertriglyceridaemia was not attenuated by fenofibrate and garlic powder.

The liver stores metabolites inclusive of minerals, vitamins, glycogen, and lipids. Thus its macro-morphometry of the liver can be influenced by the partitioning and amount of metabolites stored. London and Castonguay (2011) reported that the consumption of a high-fructose diet resulted in increased liver mass in 7-week old male Sprague Dawley rats. Importantly the high-fat high-fructose diet has been shown to trigger the development and progression of steatosis to NASH (Lozano *et al.*, 2016). In the current study, male and female rats that were fed the high-fat

high-fructose diet with fenofibrate as an intervention were significantly heavier compared to the mass of liver from rats to which other treatment regimens were administered (Tables 4.5 and 4.6). The activation of PPAR-α receptor has been shown to trigger hepatocyte proliferation and thus consequently causing hepatomegaly (Yan *et al.*, 2014). The observed increase in liver mass in the current study might be a pointer to PPAR-α receptor activation or to the potential fenofibrate-induced hepatic damage. However, we can rule out the fenofibrate-induced hepatic damage since in male and female rats none of the treatment regimens caused ballooning or caused significant differences in the non-alcoholic fatty liver disease activity scores (NAS) of the liver. Unlike fenofibrate, the oral administration of garlic powder did not cause any detrimental effect on the liver mass of female rats. Thus, these findings suggest that garlic powder can be used as ethnomedicine without inducing hepatomegaly.

Taken together, these findings infer that garlic and fenofibrate have sexually dimorphic effects with regards to high-fat high-fructose diet-induced metabolic changes.

5.4.1 Kidney mass and histology

Some phytochemicals in plant products, though commonly acclaimed for having health beneficial biological activities, can potentially damage essential organs such as kidneys (Hagiwara *et al.*, 1991). Hydroquinone, a phenolic phytochemical, has been shown to cause renal tubular cell neoplasms following its (hydroquinone) long-term administration to rats (Hagiwara *et al.*, 1991). While the glomerular filtration rate is the gold standard at assessing kidney function, surrogate markers of kidney function such as creatinine and blood urea nitrogen concentration can be used to assess kidney health (Eidi *et al.*, 2006). In the current study while in female rats' treatment regimens had no effect on the kidney mass (Table 4.8), male rats that were fed a high-fat high-fructose diet with fenofibrate as an intervention had heavier kidneys (relative to terminal body mass) compared to the kidney masses from their counterparts to which the other treatment regimens were administered (Table 4.7). These findings suggest that fenofibrate might have elicited hyperplasia or hypertrophy of the kidney cells resulting in the observed heavier masses while the high-fat high-fructose diet and garlic powder had no effect on kidney mass. The observed fenofibrate-mediated increase in kidney mass could result in pathologies that can

compromise kidney function. The similarities in the number, density and tuft area of the glomeruli, as well as that of the corpuscular and urinary spaces of the kidneys of both male and female rats, fed the control diet, the high-fat high-fructose diet and the high-fat high-fructose diet with garlic powder as an intervention (Tables 4.7 and 4.8) suggests that both the high-fat high-fructose diet and garlic powder did not elicit structural changes to the kidney at microscopic level which could be speculated to point to them (high-fat high-fructose diet and garlic powder) not causing histological changes in the rats' kidneys. Results of the current study are at variance with the reported high-carbohydrate high-fat diet-induced glomerular and tubular damage in 8-9 week old male Wistar (Panchal *et al.,* 2011).

5.4.2 Other viscera

Various chemical compounds derived from plants have been shown to affect the weight of viscera (Hagiwara *et al.,* 1991) hence viscera weight is a useful tool in the assessment of the effects these chemical compounds. In this study while in female rats treatment regimens had no effect on the mass of the small intestines (Table 4.10), the mass of the small intestines (absolute and relative to tibia length) of male rats fed a high-fat high-fructose diet and or a high-fat high-fructose diet with either fenofibrate or garlic powder as an intervention were heavier compared to that from their counterparts fed the control diet (Table 4.9). These findings suggest that the high-fat high-fructose diet-induced small intestinal cell hypertrophy and or hyperplasia which then resulted in the observed increase in the small intestines mass of male rats. The length of small and large intestines and mass of the pancreata of both male and female rats across treatment regimens were similar which suggests that the high-fat high-fructose, fenofibrate and garlic powder did not impact them (Tables 4.9 and 4.10). In female rats, while the high-fat high-fructose diet resulted in heavier large intestine mass (relative to terminal body mass) the diet (high-fat high-fructose) did not affect the mass of the large intestines (absolute and relative to tibia length) of male rats across treatment regimens (Table 4.9). The findings from the current study show that feeding a high-fat high-fructose diet in growing Sprague-Dawley rats show sexual dimorphism in regard to the diet's effect of the mass of the small and large intestines. In the current study in male rats' consumption of the high-fat high-fructose diet resulted in an increase in both visceral and epididymal fat masses (Table 4.9) suggesting that this high-calorie

diet causes adiposity. With regards to the visceral fat mass, intervening with either fenofibrate or garlic powder in growing Sprague-Dawley rats fed a high-fat high-fructose diet did not attenuate the diet-induced visceral adiposity and increased in epididymal fat. This suggests that both fenofibrate and garlic powder failed to confer protection against diet-induced adiposity. In the current study in female rats, the high-fat high-fructose diet had no effect on the absolute and relative to tibia length visceral fat mass (Table 4.10). However relative to body mass the consumption of the high-fat high-fructose diet caused increased visceral adiposity in the female rats (Table 4.10). Based on the fact that body mass centred indices of growth are prone to the effects of gut fill, and hydration status (Owens *et al.,* 1995) and the fact that long-bones respond to growth hormone in a dose-dependent manner (Eshet *et al.*, 2004), it can be argued that the observed increase in visceral adiposity in female rats relative to body mass is beset with confounding factors thus might not be a true reflection of the adiposity state. On the basis of these arguments, it can be inferred that the high-fat high-fructose diet caused adiposity only in the male rats thus such a diet might result in an increased risk in males to develop obesity-induced metabolic derangements and diseases (Tappy and Lê, 2010). Importantly, results of the current study suggest that consumption of the high-fat high-fructose diet by growing Sprague-Dawley rats affects adiposity in a sexually dimorphic manner with males more prone to the detrimental effects of the obesogenic diet. It is interesting to note that results from the current study are in tandem with the reported high-fructose diet-induced increase in visceral fat mass in humans reported by Stanhope *et al.* (2009) following a 10-week feeding intervention.

5.5 Surrogate markers of hepatic and renal health

The liver is the major site of biotransformation and metabolism of nutritional biomolecules, pharmacological agents such as antibiotics as well as the metabolism of toxins. The multiplicity of biochemical reactions that take place in liver parenchyma cells is catalysed by cell-resident enzymes, for example, the aminotransferases (Thapa and Walia, 2007). In the case of damage to liver cells, these enzymes find their way into the systemic circulation and are used as surrogate markers of liver function and are (their plasma activities) used to determine intra-hepatic and post-hepatic liver pathologies (Field *et al.*, 2008). In the current study, it was observed that feeding the high-fat high-fructose diet and its feeding with either fenofibrate or garlic powder as

an intervention did not result in increased plasma ALT or ALP activity (Tables 4.11 and 4.12). These findings suggest that these treatment regimens did not cause rupture of the liver bile duct and parenchyma cells. The findings of the current study are in variance with those by Lozano *et al.* (2016) who reported oxidative stress-induced hepatic damage in adult rats fed a high-fructose high-fat diet for 8 months. The difference in the findings of the current study and that by Lozano *et al.* (2016) could be attributed to the variance in the age and duration trials.

The gold standard in determining kidney health is to measure its glomerular filtration rate (GFR) (Xie *et al.*, 2008). However surrogate markers such as plasma creatinine and blood urea nitrogen concentration can be used in place of the GFR. Damage to kidneys results in compromised synthetic and excretory functions which manifest with increased levels of plasma creatinine and urea (Levey *et al.*, 1999). While Dissard *et al.* (2013) reported renal damage in adult mice fed a high-fructose high-fat diet for 8 months, results of the current study show that feeding a high-fructose high-fat diet to growing rats did not cause renal damage. The difference in the finding of the current and that by Dissard *et al.* (2013) could be ascribed to differences in the duration of the feeding period.

Phytochemicals in feeds are known to compromise kidney function, for example, Hagiwara *et al.* (1991) reported that the administration of caffeic acid to rats and mice cause renal tubular cell neoplasms and promoted renal carcinogenesis. In the current study, feeding of garlic powder, which contains phytochemicals, did not cause renal damage suggesting that garlic powder can be consumed without the risk of eliciting renal dysfunction.

The following chapter outlines the major conclusions drawn from the current study, study limitations and recommendations for future studies.

CHAPTER 6: CONCLUSIONS, LIMITATIONS AND RECOMMENDATIONS

6.0 Conclusions

The study evaluated the prophylactic potential of garlic powder to protect against diet-induced (high-fat high-fructose) metabolic derangements in growing rats mimicking growing children fed obesogenic diets.

In the current study, the consumption of a high-fat high-fructose diet elicited both metabolic derangements (decreased haematocrit, increased plasma triglyceride concentration and visceral adiposity) and histo-morphometric changes (hepatic inflammation and steatosis) in a sexually dimorphic manner. The high-fat high-fructose diet decreased PCV of male rats but had no effect on the PCV of female rats. Additionally, the high-fat high-fructose diet resulted in hepatic inflammation and steatosis in female rats (which was protected by garlic powder and fenofibrate) and not in male rats. Garlic powder and fenofibrate protected the rats against the high-fat high-fructose diet-induced metabolic derangements in a sexually dimorphic manner. The use of either fenofibrate and or GP might cause derangement of blood glucose homeostasis in growing male children since they compromised post-prandial glycaemic control in growing male rats fed an obesogenic diet. In growing children, garlic powder and fenofibrate can potentially be used to protect against some components of the HFHF diet-induced MD but should be used with caution as they might cause adverse health outcomes.

6.1 Limitations

In interrogating the potential proactive effect of garlic powder against diet-induced metabolic derangements, the current study did not make use of molecular techniques which could have allowed for better insight of the mechanisms at play.

6.2 Recommendations

It is thus recommended that future studies consider the use of molecular techniques in order to increase the depth of understanding the findings and also possible mechanisms associated with the findings. It is also, therefore, recommended that future studies make use of purified

phytochemicals derived from garlic in a bid to find natural prophylactic agents against metabolic derangements.

CHAPTER 7: REFERENCES

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APPENDICES

Appendix 1: Animal ethical clearance certificate

STRICTLY CONFIDENTIAL

ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2016/03/13/B

APPLICANT: Ms T Ngoetsana

SCHOOL: Physiology
DEPARTMENT:
LOCATION:

PROJECT TITLE: The effect of garlic (Allium sativum) powder on growing Sprague Dawley rats fed a high-fat high-fructose diet

Number and Species

50 male 21 days old Sprague Dawley rats and 50 female 21 day old Sprague Dawley rats

Approval was given for the use of animals for the project described above at an AESC meeting held on 2016/03/29. This approval remains valid until 2018/04/03.

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:

Signed: _____ (Chairperson, AESC) Date: 1 August 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: 2 August 2016

cc: Supervisor: Dr E Chivandi
Director: CAS

Works 2000/lain0015/AESCCert.wps

Appendix 2: Elisa method

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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℃. 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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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 transferpettor, 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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