EFFECTS OF METHANOLIC EXTRACT OF *MORINGA OLEIFERA* LEAVES ON FRUCTOSE-INDUCED METABOLIC DYSFUNCTION IN GROWING SPRAGUE DAWLEY RATS

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DECLARATION

I, Nasiru Muhammad, declare that the work in this dissertation is my own, except where others have helped as acknowledged. This dissertation is being submitted for the degree of Master of Science in Medicine in the Faculty of Health Sciences at the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any degree or examination at any University. All the experimental procedures used in this dissertation were approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (AESC number: 2015/11/51/B).

Nasiru Muhammad

Signed on the 31st day of August, 2017

DEDICATION

This work is dedicated with love and affection to my mother, Hajiya Aishatu Chirido, my father Mallam Muhammad Dan Inne, and my brother Alhaji Mustapha Muhammad.

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ABSTRACT

Excess dietary fructose intake has been associated with an increase in metabolic disorders. Traditionally, these disorders are managed by physical exercise, lifestyle modification, and by conventional drug therapy. A significant proportion of the population also depends on the therapeutic/prophylactic properties of natural plants for their medical problems. The tree *Moringa oleifera* is well recognized for its medicinal and nutritional properties. The plant is said to possess antiobesity, antilipidaemic, antidiabetic and hypotensive effects amongst other medicinal properties.

Most previous studies that explored the effects of *Moringa oleifera* on metabolism used adult male experimental animal models without considering adult female and young growing animal models, despite the increasing prevalence of metabolic syndrome in females and growing children. This study thus investigated the impact of a methanolic extract of *Moringa oleifera* leaves on fructose-induced metabolic dysfunction in growing Sprague Dawley rats of both sexes.

One hundred and two (102), 21 day old, weaned male and female pups were randomly allocated to six groups that were sex matched. All groups received standard commercially sourced rat chow *ad libitum* throughout the study. In addition, Group I (negative control) received tap water for drinking and plain gelatine cubes. Group II received 20% fructose solution as drinking fluid and plain gelatine cubes. Group III received 20% fructose solution as their drinking fluid and 400 $mg.kg^{-1}$ body weight of methanolic extract of *Moringa oleifera* leaves suspended in gelatine cubes. Group IV received 20% fructose solution as their drinking fluid and 100 $mg.kg^{-1}$ body weight of fenofibrate (positive control) suspended in gelatine cubes. Group V received 400 $mg.kg^{-1}$ body weight of the methanolic leaf extracts of *Moringa oleifera* in gelatine cubes and had plain drinking water. Group VI received 100 $mg.kg^{-1}$ body weight of fenofibrate in gelatine cubes and had access to plain drinking water.

After 10 weeks of the interventions, the rats were euthanased by anaesthetic overdose following an overnight fast; and samples of blood and tissue were collected. The outcomes of the interventions on growth performance, morphometry of the gastro-intestinal tract organs, circulating metabolites, adiposity, liver lipid accumulation and general health markers were assessed.

Data were expressed as mean ± standard deviation and analyzed by one-way or two-way analysis of variance (ANOVA) depending on the variables. The statistical significance of analyzed values was set at ≤ 5%.

Administration of 20% fructose solution significantly elevated hepatic lipid content in both sexes ($P<0.0001$) and the concentration of circulating triglycerides in female rats ($P<0.0001$) compared with negative controls. These lipid elevations were prevented by the administration of 400 $mg.kg^{-1}$ body weight of methanolic extract of *Moringa oleifera* leaves and by 100 $mg.kg^{-1}$ body weight of fenofibrate ($P\leq0.05$). The effect of fenofibrate was more pronounced than that of *Moringa*. Fenofibrate treated groups (both sexes) had hepatomegaly ($P<0.0001$), higher fasting blood glucose (FBG) ($P<0.0001$), higher alkaline phosphatase activity in plasma ($P<0.05$) and lower ($P<0.05$) epididymal fat relative to tibial length (males) compared with the other treatment groups. The plasma triglycerides and cholesterol levels were higher in females than in males ($P<0.05$). The absolute and relative visceral fat pad masses were also higher in females ($P<0.05$). There were no significant differences in the hepatic lipid content and creatinine levels between the two sexes ($P>0.05$). However, male rats had significantly higher levels of FBG, liver enzymes (ALT and ALKP), blood urea nitrogen (BUN), urea to creatinine ratio and higher organ morphometry than their corresponding females ($P<0.0001$).

No adverse effects were observed with fructose or *Moringa* on growth, organ morphometry, determinants of metabolic dysfunction and surrogate markers of general health. However, hepatomegaly was observed in fenofibrate treated groups ($P<0.0001$).

In the present study, sex differences were observed in the metabolic responses of growing Sprague Dawley rats to a high-fructose diet. In addition, the methanolic extract of *Moringa oleifera* leaves was beneficial in preventing the hypertriglyceridaemia and abnormal deposition of hepatic lipids in high-fructose fed animals. However, the extract was not effective in preventing fructose-induced visceral obesity in male animals. The use of methanolic leaf extracts of *Moringa oleifeira* should be further explored as a possible candidate prophylactic intervention in the fight against the global epidemic of diet induced metabolic dysfunction.

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LIST OF ABBREVIATIONS

α: Alpha

β: Beta

°C: Degrees celsius

%: Percent

μg.g^{-1}: Microgram per gram

μU.ml^{-1}: Micro unit per millilitre

AACE: American Association of Clinical Endocrinologists

Acetyl-CoA: Acetyl coenzyme A

Acyl-CoA: Acyl Coenzyme A

AEMO: Aqueous extract of *Moringa oleifera*

AESC: Animal Ethics Screening Committee

AGE: Advanced glycation end-product

ALKP: Alkaline phosphatase

ALT: Alanine aminotransferase

ANOVA: Analysis of variance

AST: Aspartate aminotransferase

ATP: Adenosine triphosphate

ATP III: Adult Treatment Panel three

BMG: Body mass gain

BMI: Body mass index

BUN: Blood urea nitrogen

C: Negative control group

Ca^{2+}: Calcium ion

CAS: Central Animal Services

DNA: Deoxy-ribonucleic acid

EGIR: European Group for the study of Insulin Resistance

ELISA: Enzyme Linked Immuno-Sorbent Assay

FBG: Fasting blood glucose

FFAs: Free fatty acids

FNF: The group on fenofibrate alone

F+H: The group on combined fenofibrate and fructose

g: Gram

grTL: Gram relative to tibial length

GFR: Glomerular filtration rate

GIT: Gastro-intestinal tract

GLUT: Glucose transporter

GSH: Glutathione

H: The group on fructose solution alone

H_0: Null hypothesis

H_1: Alternative hypothesis

HDL: High density lipoprotein

HDL-c: High density lipoprotein cholesterol

HFCS: High fructose corn syrup

HMG-Co-A: 3-hydroxy-3-methylglutaryl-coenzyme A

HOMA-IR: Homeostatic model of insulin resistance

IDF: International Diabetes Federation

IL-6: Interleukin six

kg: Kilogram

LDL: Low density lipoprotein

LDL-c: Low density lipoprotein cholesterol

L.I: Large intestines

MDA: Malondialdehyde

MEMO: Methanolic extract of *Moringa oleifera*

mg.dl: Milligram per decilitre

mg.kg^{-1}: Milligram per kilogram

mm: Millimetre

ml: Millilitre

Mo: The group on *Moringa oleifera* alone

MOLP: *Moringa oleifera* leaf powder

M+H: The group on combined *Moringa* and fructose

MS: Metabolic syndrome

Na^+: Sodium

NACEP: National Cholesterol Education Program

NAFLD: Non-alcoholic fatty liver disease

NASH: Non-alcoholic steatohepatitis

ng.ml^{-1}: Nanogram per millilitre

NO: Nitric oxide

P: Phosphate

pH: A measure of acidity or alkalinity

PPAR: Peroxisome proliferator-activated receptor

Q3G: Quercetin-3-glycoside

ROS: Reactive oxygen species

SD: Sprague Dawley

S.I: Small intestines

STZ: Streptozotocin

TAG: Triacylglycerol

TGs: Triglycerides

TNF: Tumour necrosis factor

Ur:Cr^{-1}: Urea to creatinine ratio

VLDL: Very low density lipoprotein

VLDL-c: Very low density lipoprotein cholesterol

WC: Waist circumference

WHO: World Health Organization

CHAPTER ONE- INTRODUCTION

1.1 Preface

Metabolic syndrome (MS) is a clinical condition that comprises obesity, lipid abnormalities, increased blood sugar, insulin resistance and increased blood pressure.

Increased consumption of diets high in carbohydrate and fat has increased the prevalence of MS in adults, adolescents and children. Poor lifestyle choices also contribute to the increased prevalence in these age groups. In addition, high dietary fructose intake predisposes to the development of MS in all ages.

Conventional synthetic agents used in managing the components of metabolic syndrome like fenofibrate, are very expensive; as such cannot be afforded by most communities in developing countries. The agents are also associated with adverse effects.

Most communities in developed and developing countries use plants in solving their medicinal problems, including the risk factors associated with metabolic syndrome. *Moringa oleifera Lam.* (drumstick tree) is one of such plants being used in managing different ailments. The plant is said to possess hypoglycaemic, hypolipidaemic and antiobesity properties.

Most studies have used adult male rats to evaluate the medicinal potential of *Moringa oleifera*. The studies also tended to focus on a single risk factor of metabolic syndrome. As such, there is need to explore the medicinal potential of *Moringa oleifera* in managing holistically the components of metabolic syndrome concomitantly and taking into consideration growing individuals of both sexes.

This study investigated the effects of methanolic leaf extract of *Moringa oleifera* on fructose-induced metabolic dysfunction in growing male and female Sprague Dawley rats as an experimental animal model for growing children at risk of high fructose induced metabolic dysfunction.

The dissertation broadly comprises the following: an introductory chapter, a chapter detailing the materials and methodology employed to meet the objectives, a chapter with the results, a chapter providing a critical contextualized discussion of the findings, and finally a chapter expounding the conclusions and proffering some recommendations

arising from the study outcomes. A list of references is also included. Supplementary information is included as appendices.

1.2 Introduction

The combination of central obesity, lipid abnormalities, increased blood sugar, insulin resistance and increased blood pressure is termed metabolic syndrome (MS) (Mamikutty *et al.*, 2014; Nakagawa *et al.*, 2006). Central obesity and insulin resistance are associated risk factors for cardiovascular disease and diabetes (Ghezzi *et al.*, 2012). The presence of obesity with diabetes in an individual occurs in both developed and developing countries, and these conditions are among the major public health problems faced globally (Zimmet *et al.*, 2005). Obesity and diabetes have been attributed to the consumption of diets high in carbohydrate and fat (Mamikutty *et al.*, 2014), and poor lifestyle options (Nakagawa *et al.*, 2006) amongst other factors.

Globally, MS has been defined in many ways by different organizations using different criteria (Mamikutty *et al.*, 2014). These organizations include the European Group for the study of Insulin Resistance (EGIR), the World Health Organization (WHO), the National Cholesterol Education Program (NCEP) ATP III (Adult Treatment Panel III) (Kengne *et al.*, 2012) and the American Association of Clinical Endocrinologists (AACE) (Zimmet *et al.*, 2005). The multiplicity of definitions resulted in difficulty undertaking comparable clinical and epidemiological research (Zimmet *et al.*, 2005). This led to the development of a new definition by the International Diabetes Federation (IDF), collaborating with representatives from previous organizations who came from all regions of the world. The new definition is known as “harmonized criteria” (Mamikutty *et al.*, 2014; Zimmet *et al.*, 2005) and has made it clinically useful, and epidemiologically possible to compare research regarding metabolic syndrome between countries as well as between different population groups (Zimmet *et al.*, 2005).

The IDF worldwide definition of MS emphasizes on the presence of an ethnicity-specific parameter, which is central obesity (waist circumference), in addition to any two of: "hypertriglyceridaemia (TGs) (≥150 $mg.dl^{-1}$), low high density lipoprotein cholesterol (HDL-c) (<40 $mg.dl^{-1}$ in males and <50 $mg.dl^{-1}$ in females), elevated fasting plasma glucose (≥100 $mg.dl^{-1}$) and/or raised blood pressure (≥130 mmHg systolic or ≥85 mmHg diastolic)” (Cirillo *et al.*, 2006; Zimmet *et al.*, 2005).

Metabolic syndrome is caused by many factors which include genetic make-up and lifestyle options; poor dietary habits and a decrease in physical activity have increased the risk of developing the syndrome (Angelova and Boyadjiev, 2013).

Worldwide there is an increasing prevalence of metabolic syndrome (Mamikutty *et al.*, 2014; Cirillo *et al.*, 2006). Globally, it is estimated at about 20 to 25 percent (%) in the adult population (Mamikutty *et al.*, 2014), and 3 to 4 percent in children and adolescents (Cruz and Goran, 2004).There is usually a lower prevalence in men than in women irrespective of the criteria used to define the condition (Kengne *et al.*, 2012). The prevalence of MS in rural populations in South Africa was found to be greater in women (25%) than in men (10.5%) (Motala *et al.*, 2011). In a Kenyan urban community, it was 40.2% in women and 29% in men (Kaduka *et al.*, 2012). The overall prevalence in West Africa was 42% in women and 19% in men (Kaduka *et al.*, 2012). Components of metabolic syndrome are now increasingly being observed in children and adolescents (Pandita *et al.*, 2016). Type 2 diabetes is being reported in these age groups in many countries (Zimmet *et al.*, 2005), with nearly equal prevalence between males and females (Cirillo *et al.*, 2006). The prevalence of MS is estimated at about 30 percent in children with high body mass index (BMI) (Cruz and Goran, 2004). This increasing prevalence of MS has been attributed as is the case in adults, to the consumption of diets high in carbohydrate and fat (Mamikutty *et al.*, 2014), and poor lifestyle options (Nakagawa *et al.*, 2006).

Metabolic syndrome is associated with the onset of diabetes mellitus (type 2) and cardiovascular diseases (Cirillo *et al.*, 2006). Apart from the high rates of morbidity and mortality associated with diabetes mellitus, micro- and macro- vascular complications can lead to limited or reduced daily activity translating to loss of productivity (Toma *et al.*, 2015). Atherosclerosis of the heart vessels has been documented in patients with metabolic syndrome (Cruz and Goran, 2004). It has also been shown that chronic kidney disease and microalbuminuria are associated with metabolic syndrome (Cirillo *et al.*, 2006). Death secondary to cardiovascular complications increases by 100% within five to ten years when two or more components of metabolic syndrome are present (Mamikutty *et al.*, 2014).

The increase in obesity and metabolic disorders has been associated with an increase in fructose intake in the past few years (Lê and Tappy, 2006).

1.3 Fructose and metabolic syndrome

Fructose is a simple sweet tasting sugar that is naturally present in fruits and honey (Khitan and Kim, 2013). It has the same chemical formula with glucose, $C_6H_{12}O_6$; but differs structurally with glucose in that glucose has an aldehyde moiety on the first carbon while fructose has a keto group on carbon number two (Tappy, 2012).

Sucrose and High Fructose Corn Syrup (HFCS) are the major sources of dietary fructose globally (Khitan and Kim, 2013). Consumption of fructose increased following introduction of new beverages like soft drinks (Tappy and Lê, 2010), tea, coffee and cocoa; and production of chocolates and ice-creams (Tappy, 2012).

Excess dietary fructose intake causes liver insulin resistance and increased body fat deposition; this predisposes to the pathogenesis of metabolic syndrome (Tappy *et al.*, 2010). There is an association between obesity and insulin resistance that leads to an inability to tolerate glucose and hyperinsulinaemia (Divi *et al.*, 2012).

Liver gluconeogenesis is enhanced in the presence of excess concentrations of free fatty acids (FFAs) which can be seen when there is an increase in fructose metabolism (Divi *et al.*, 2012) leading to hyperglycaemia (Elliott *et al.*, 2002). Fructose mediates vascular dysfunction by activating the adipocyte-renin-angiotensin II system (Khitan and Kim, 2013). As a result, adipocytes release cytokines like interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α)(Khitan and Kim, 2013). These cytokines affect beta (β) cells of the pancreas and may lead to insulin resistance (Khitan and Kim, 2013). Insulin is involved in metabolism of carbohydrates, lipids and proteins (Wilcox, 2005). Leptin, an adipokine, is also a regulator of energy balance and together with insulin, leptin decreases energy intake and increases energy utilization (Rezvani *et al.*, 2013). Leptin also favours lipid deposition in peripheral tissues through stimulation of fatty acid oxidation thereby affecting insulin sensitivity (Rezvani *et al.*, 2013). It has been shown that intake of fructose-rich diets decreases 24-hour plasma insulin and leptin levels (Bray *et al.*, 2004). Insulin secretion is not stimulated by fructose (Khitan and Kim, 2013). In cases of sustained fructose diet consumption, decreased leptin concentrations

translate into excessive caloric intake, and this leads to obesity and weight gain (Khitan and Kim, 2013).

Excess dietary fructose intake was also found to elevate serum uric acid which causes renal vasoconstriction; this eventually leads to elevated systemic blood pressure (Khitan and Kim, 2013).

Fructose is metabolized in the liver differently from glucose (Bray *et al.*, 2004). Glucose metabolism is insulin dependent and is regulated by negative feedback mechanism depending on the energy requirements of the cells (Tappy and Lê, 2010), where excess glucose is stored as glycogen (Elliott *et al.*, 2002) (Figure 1.1). The regulation is effected at the level of an enzyme phosphofructokinase which is inhibited by adenosine triphosphate (ATP) and citrate (Tappy and Lê, 2010). Unlike glucose, entry of fructose into cells is independent of insulin, and is a function of the glucose transporter-5 (GLUT-5)(Bray *et al.*, 2004). The transporter is absent in β-cells of the pancreas and in the brain, thereby limiting fructose entry into these tissues (Bray *et al.*, 2004). As a consequence, there is an inability of the fructose to send satiety messages to the brain (Bray *et al.*, 2004). Although small amounts of fructose may be metabolized within the gastro-intestinal tract (GIT) by enterocytes, the bulk of it is absorbed into the portal vein from the GIT (Tappy, 2012). While in the gut, it is transported into the enterocytes by GLUT-5, after which it is then transported into the hepatocytes by glucose transporter-2 (GLUT-2) (Tappy and Lê, 2010). When fructose enters the liver cells, it is phosphorylated to ATP (through the action of an enzyme called fructokinase) to form fructose-1-phosphate (Elliott *et al.*, 2002) (Figure 1.1). The fructose-1-phosphate is acted upon by aldolase to formglyceraldehyde and dihydroxyacetone phosphate known as triose-phosphates (Elliott *et al.*, 2002). These trioses can be converted to glyceraldehyde-3-phosphate, and at the same time they are the backbone in the formation of phospho-lipids, triacylglycerol (TAG) and fatty acids (Bray *et al.*, 2004). Fructose metabolism is not regulated and thus produces more lactate than does glucose metabolism, and this (lactate) serves as a major source of acetyl-CoA leading to the formation of TAGs and VLDL (Elliott *et al.*, 2002).

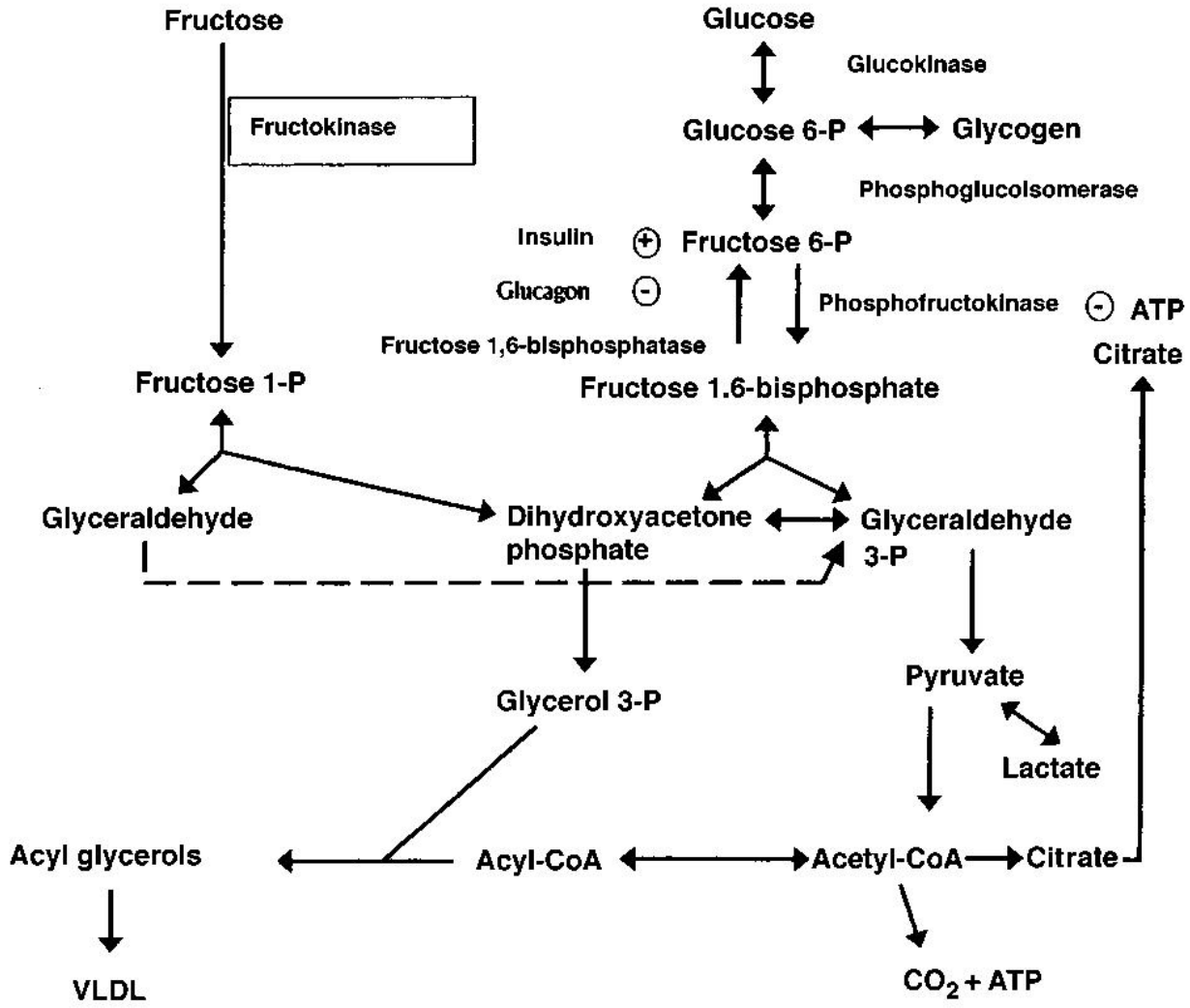

Figure 1.1: Hepatic metabolism of fructose and glucose (Elliott *et al.*, 2002). Abbreviations: P= Phosphate; Acetyl-CoA= Acetyl Coenzyme A; Acyl-CoA= Acyl Coenzyme A; CO_2= Carbon dioxide; ATP= Adenosine triphosphate; VLDL= Very low density lipoprotein; (+)= Stimulatory effect; (-)= inhibitory effect.

This biochemical process shows that excess dietary fructose enhances production of TAGs more than does glucose (Bray *et al.*, 2004). This is because fructose enters the glycolytic pathway through bypassing the rate limiting step that is catalyzed by the enzyme phosphofructokinase; and fructose also interferes with the removal of TAGs that are usually synthesized and eliminated by stimulation from glucose (Divi *et al.*, 2012).

Hyperglycaemia may result from glucose that might be produced secondary to fructose metabolism. This is due to fructose-induced insulin resistance that inhibits the ability of insulin to metabolize the glucose (Divi *et al.*, 2012).

Lifestyle modification and increased physical activity are the initial interventions in managing MS, which could be supplemented by drug therapy (Ahmed *et al.*, 2014; De Fronzo and Ferrannini, 1991). The drugs include fibrates, statins and niacin that are used to manage lipid abnormalities (Chapman *et al.*, 2010), and metformin and sulfonylureas that are used to lower blood glucose levels (Florez *et al.*, 2014; Thakkar *et al.*, 2013).

Worldwide there is interest in using plant-derived or herbal medicines which are 'natural' and thus thought to be safer than the 'synthetic medicinal agents(Ekor, 2014). More than 80 percent of individuals worldwide use herbal medicinal products even though the safety of most of these herbal products is not well established (Ekor, 2014). As such there has been a great interest in ethnomedicines by academic researchers worldwide (Fakurazi *et al.*, 2008a; Erasto *et al.*, 2005). There is therefore a need to explore the medicinal potential of less expensive natural agents with potentially minimal adverse effects but also providing holistic medication in the management of MS (Ndong *et al.*, 2007; Erasto *et al.*, 2005). *Moringa oleifera* (drumstick tree) is one of such plants used in managing the components of MS (Ahmed *et al.*, 2014), and it is thus the plant of interest in this study.

1.4 *Moringa oleifera* plant

Moringa oleifera Lam. (Moringa pterygosperma Gaertn), belongs to a family called *Moringaceae* of the angiosperm plants (Mbikay, 2012). It is also known as ben oil tree, drumstick tree or horse radish tree (Stohs and Hartman, 2015). It is native to India but presently naturalized in many parts of the world including Africa (Mbikay, 2012). *Moringa oleifera* plant is widely cultivated in Asia, tropical Africa and tropical America (Sabale *et al.*, 2008). The cultivation of *Moringa* plant is increasing in the tropical regions of the world because the plant is able to propagate both sexually and asexually, tolerate small soil nutrients (Adedapo *et al.*, 2009) and withstand drought conditions (Anwar *et al.*, 2007). The plant grows well in tropical regions and can reach up to 10 meters in height

(Sabale *et al.*, 2008) within regions with an annual rainfall that ranges between 250 mm to 3000 mm (Anwar *et al.*, 2007).

Figure 1.2: *Moringa oleifera* plant at Agricultural Research Council Research Farm-Roodeplaat, Pretoria, Republic of South Africa (Photo taken by Professor Eliton Chivandi on 13/02/2017).

Moringa oleifera is reported to possess medicinal properties (Sánchez-Machado *et al.*, 2010). Different parts of *Moringa oleifera* including the leaves, flowers, immature pods,

seeds, bark and the roots, have been widely used as food ; as well as in treating different ailments (Zvinorova *et al.*, 2015; Sangkitikomol *et al.*, 2014). Various preparations of these parts like aqueous, alcohol or hydroalcohol extracts have also been used in animal studies where they have been shown to possess hypoglycaemic, hypolipidaemic and antiobesity properties (Stohs and Hartman, 2015) mostly in adult male rats. The tree extracts also exhibit antihypertensive (Faizi *et al.*, 1998), antioxidant (Gupta *et al.*, 2012) and antibiotic (Nikkon *et al.*, 2003) properties.

1.4.1 Phytochemical composition of *Moringa oleifera*

The phytochemical composition of *Moringa oleifera* depends on the plant parts analyzed (Sánchez-Machado *et al.*, 2010) and the solvent used in the extraction process (Kasolo *et al.*, 2010). The *Moringa oleifera* leaves are rich in β-carotene, protein, calcium, potassium (Divi *et al.*, 2012), starch, iron and vitamins A, B and C (Zvinorova *et al.*, 2015). *Moringa oleifera* leaves were shown to contain dry weight of 18.7 microgram per gram ($\mu g.g^{-1}$) β-carotene, 22.9 percent (%) protein, 14000 $\mu g.g^{-1}$ calcium, 370 $\mu g.g^{-1}$ iron, and 81.2 $\mu g.g^{-1}$ lutein (Zvinorova *et al.*, 2015). The leaves of the plant are also rich in flavonoids, phenolics and carotenoids (Stohs and Hartman, 2015; Anwar *et al.*, 2007). Niazinin A, niazinin B, niazinin A+B and niazimicin were also isolated from the ethanolic leaf extract of *Moringa oleifera* (Anwar *et al.*, 2007). The *Moringa* seed oil contains sterols which include β-sitosterol, campesterol and stigmasterol (Anwar *et al.*, 2007). The plant also contains nitrile mustard oil glycosides, thiocarbamate glycosides (Divi *et al.*, 2012) and tocopherols (alpha, beta and gamma) (Anwar *et al.*, 2007). Fatty acids which include linolic acid, linolenate, oleic acid, hexadecanoic (palmitic) acid and arachidonate are also found in different parts of the plant (Sánchez-Machado *et al.*, 2010).

1.4.2 Hypolipidaemic and antiobesity properties of *Moringa oleifera*

It has been reported that methanolic leaf extract of *Moringa oleifera* decreases serum cholesterol concentrations, phospholipids, triacylglycerol (TAG), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) (Jain and Surana, 2015). It also decreases atherogenic index and faecal excretion of cholesterol (Jain and Surana, 2015). A decrease in cholesterol to phospholipid ratio and lipid profile of the liver has also been documented (Anwar *et al.*, 2007).

The lipid lowering effect of *Moringa oleifera* might be attributed to the presence of β-sitosterol (Divi *et al.*, 2012; Ghasi *et al.*, 2000) which has the same structure with cholesterol, except for an ethyl group that is substituted at carbon 24 of its side chain. As a result, it indirectly decreases cholesterol levels by lowering plasma LDL-cholesterol concentrations which is considered as the primary target of hypolipidaemic treatment (Ahmed *et al.*, 2014).

Both ethanolic and methanolic extracts of *Moringa oleifera* leaves have inhibitory effects on 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-Co-A) reductase. This enzyme is a catalyst for the rate limiting process in the biosynthesis of cholesterol (Ahmed *et al.*, 2014). An inverse relationship exists between the HMG-Co-A/mevalonate ratio and the activity of HMG-Co-A reductase; the higher the ratio the less the enzyme activity (Ahmed *et al.*, 2014; Jain *et al.*, 2010). An increase in this ratio was observed by Jain *et al.* (2010) after methanolic extract of *Moringa* was given to obese Wistar rats for 30 days, which depressed the activity of the enzyme leading to hypocholesterolemia.

Ahmed *et al.* (2014)showed that in rats that were fed a high cholesterol diet, there was decrease in abdominal circumference and body mass index (BMI) following administration of 600 $mg.kg^{-1}$ body weight of ethanolic extract of *Moringa oleifera* leaves for 12 weeks. This was attributed to inhibition of dietary fat utilization by saponins and tannins which eventually manifested as poor growth performance (Ahmed *et al.*, 2014). The study also documented elevated serum high density lipoprotein cholesterol (HDL-c) concentration which is beneficial for health outcomes (Jain *et al.*, 2010).

Bais *et al.* (2014)reported similar findings after using 400 $mg.kg^{-1}$ body weight of methanolic extract of *Moringa oleifera* (MEMO) leaves for seven weeks on rats with obesity induced by a high fat-diet. Thus *Moringa oleifera* has great therapeutic potential in the management of obesity. Obesity is frequently linked with diabetes mellitus (Ahmed *et al.*, 2014).

1.4.3 Hypoglycaemic and antidiabetic properties of *Moringa oleifera*

Hyperglycaemia may be a consequence of several factors including defects in the synthesis or action of insulin leading to various complications that affect cardiovascular, renal or neurological systems (Jaiswal *et al.*, 2009).

The hypoglycaemic effects of *Moringa oleifera* are believed to be due to phytochemicals present in the plant. These phytochemicals include N-Benzyl thiocarbamates and benzyl esters which stimulate insulin release from β-cells of the pancreas (Gupta *et al.*, 2012; Jaiswal *et al.*, 2009). Flavonoids like quercetin-3-glycoside (Q3G) and fiber which are present in *Moringa oleifera* leaves, delay gastric emptying leading to low postprandial glycaemia as well as reduced postprandial small intestinal glucose absorption (Ndong *et al.*, 2007). The flavonoid (Q3G) also inhibits glucose uptake by inhibiting both sodium (Na^+) dependent and Na^+ independent glucose uptake at the intestinal mucosa (Ndong *et al.*, 2007).

Jaiswal *et al.* (2009),observed a decrease in blood glucose levels following administration of aqueous leaf extracts of *Moringa oleifera* (AEMO) in rats with damaged islets, suggesting that there was direct peripheral tissues utilization of glucose. This also indicates that *Moringa* might influence the synthesis as well as the action of insulin (Gupta *et al.*, 2012; Ndong *et al.*, 2007). The study also observed that the AEMO leaves used on hyperglycaemic rats decreased blood glucose levels more than the positive control drug (Glipizide) (Jaiswal *et al.*, 2009).

1.4.4 Hypotensive and cardioprotective properties of *Moringa oleifera*

The hypotensive effect of seeds and leaves of *Moringa oleifera* is ascribed to their content of β-sitosterol, methyl p-hydroxybenzoate, thiocarbamate and isothiocyanate glycosides. This was observed following administration of both ethanolic and aqueous extracts on Wistar rats (Anwar *et al.*, 2007; Faizi *et al.*, 1998). Ethanolic extract of *Moringa oleifera* leaves contains niazinin A, niazinin B, niazinin A+B and niazimicin; these compounds have hypotensive activity in rats by antagonising calcium (Ca^{2+}), which is a potent inotropic agent (Anwar *et al.*, 2007). Anwar *et al.* (2007) also documented the diuretic effect of *Moringa oleifera*, which contributes to lowering the blood pressure.

The Atherogenic index indicates fatty infiltration in the heart, liver or kidneys and thus a pointer to the risk of developing cardiovascular, hepatic or renal diseases (Jain *et al.*, 2010). The index is determined using Friedewald formula as follows (Bais *et al.*, 2014):

“Atherogenic index = (Total cholesterol − HDL-c) ÷ HDL-c”

This index was decreased following administration of MEMO for 30 days to obese Wistar rats (Jain *et al.*, 2010).

Thus, the ability of *Moringa oleifera* to possess hypolipidaemic, hypotensive and diuretic properties makes it a highly cardioprotective plant (Anwar *et al.*, 2007).

1.4.5 Antioxidant properties of *Moringa oleifera*

A notable factor in the pathogenesis of diabetes and cardiovascular diseases is oxidative stress arising from free radicals and reactive oxygen species (ROS) (Mbikay, 2012). Cells maintain their functional integrity against oxidative damage by decreasing ROS production and increasing free radicals scavenging capacity; this they achieve through the actions of antioxidant enzymes in the liver, as well as by the levels of reduced glutathione (GSH) (Mbikay, 2012). The hepatic antioxidant enzymes include “catalase, superoxide dismutase, glutathione peroxidase” (Mbikay, 2012), “glutathione transferase and glutathione reductase” (Sangkitikomol *et al.*, 2014). The antioxidant pool of the body is also influenced by dietary intake of substances with antioxidant properties (Sangkitikomol *et al.*, 2014).

Leaves, flowers and seeds of *Moringa oleifera* possess antioxidant properties which are believed to be chiefly due to the flavonoid content of the plant (Mbikay, 2012). Both aqueous and ethanolic extracts of *Moringa oleifera* inhibit oxidative damage in normal and diabetic subjects (Stohs and Hartman, 2015). These extracts achieve this through increasing free radical scavenging, increasing the levels of antioxidant enzymes , increasing hepatic glutathione and decreasing peroxidation of lipids (Stohs and Hartman, 2015).

Ahmed *et al.* (2014) documented a decrease in serum malondialdehyde (MDA) levels (a lipid peroxidation product) following administration of AEMO to obese adult female Wistar rats for 30 weeks. The study also observed a significant decrease in nitric oxide (NO) due to inhibition of nitric oxide synthase that catalyzes the production of NO. The overall effect was a decrease in oxidative stress associated with these oxidative products.

Through its antioxidant properties, *Moringa* extracts also inhibit the formation of advanced glycation end-product (AGE) (Sangkitikomol *et al.*, 2014). AGEs are lipid

peroxidation products which are shown to worsen oxidative stress (Sangkitikomol *et al.*, 2014).

1.4.6 Hepatoprotective properties of *Moringa oleifera*

Liver damage can be protected through antioxidant effects of *Moringa* extracts. This was documented by Jaiswal *et al.* (2009) following administration of AEMO leaves on normal and diabetic rats. In both subjects, there was increase in actions of liver antioxidant enzymes and glutathione, and decrease in lipid peroxidation. Quercetin, a flavonoid present in both aqueous and methanolic extracts of *Moringa* , possesses a significant hepatoprotective effect (Anwar *et al.*, 2007).

Carbon tetrachloride, acetaminophen (paracetamol) and anti-tuberculosis drugs (Isoniazid, pyrazinamide and rifampicin) cause hepatic toxicity by increasing the levels of hepatic enzymes aspartate aminotransaminase (AST), alanine aminotransaminase (ALT) and alkaline phosphatase (ALKP), bilirubin and lipid peroxidation products (Stohs and Hartman, 2015). These agents also tend to decrease the levels of antioxidant enzymes (Stohs and Hartman, 2015). Administration of *Moringa oleifera* extract to rats decreased the liver enzymes activity, bilirubin level and the formation of lipid peroxidation products; and re-established normal concentrations/activities of antioxidant enzymes (Fakurazi *et al.*, 2008a; Pari and Kumar, 2002) and GSH (Fakurazi *et al.*, 2008b).

1.4.7 Antimicrobial properties of *Moringa oleifera*

Crude and ethanolic extracts of *Moringa oleifera* root barks have been shown to possess significant antibacterial and antifungal activity (Anwar *et al.*, 2007; Nikkon *et al.*, 2003).

The juice of fresh leaves of *Moringa oleifera* is active against *Micrococcus pyogens, Escherichia coli* and *Bacillus subtilis* (Siddhuraju and Becker, 2003).

The antimicrobial action of *Moringa* is attributed to the alkaloids, terpenoids and steroids in the plant extracts (Kasolo *et al.*, 2010). The alkaloids (moringine and moringinine) are nitrogen containing compounds that interfere with the deoxy-ribonucleic acid (DNA) of the pathogens (Kasolo *et al.*, 2010). Pterygospermin is reported to have a strong antibacterial activity against *Helicobacter pylori* (Fahey, 2005).

1.4.8 Antineoplastic properties of *Moringa oleifera*

Studies have shown that *Moringa oleifera* extracts are also effective against carcinogenesis. Aqueous leaf extracts of *Moringa oleifera* are effective against lung neaoplasia by increasing the ROS level, inducing programmed cell death (Tiloke *et al.*, 2016), and interfering with the growth of tumour cells (Tiloke *et al.*, 2013). Niazimicin, a chemical constituent of *Moringa* seeds, has anticancer activity against skin and hepatic tumours (Stohs and Hartman, 2015). This is achieved through its anti-tumour promotion effect (Anwar *et al.*, 2007). Thiocarbamates and isothiocyanates isolated from aqueous leaf extracts of *Moringa oleifera* inhibited Epstein-Barr virus-induced tumour promotion (Stohs and Hartman, 2015; Fahey, 2005).

1.4.9 Other properties of *Moringa oleifera*

A relationship exists between inflammatory processes and the pathophysiology of the components of MS (Kerner *et al.*, 2005). Studies have shown that *Moringa oleifera* extracts have anti-inflammatory activity in rats and mice (Gupta *et al.*, 2012) by inhibiting tumour necrosis factor alpha (TNF-α) (Ahmed *et al.*, 2014).

MEMO leaves were documented to possess anti-ulcer activity in rats (Siddhuraju and Becker, 2003). Through its antibiotic effect against *Helicobacter pylori*, which is culpable in the aetiology of gastric ulcers, MEMO leaf extract has been used to treat gastritis, gastric and duodenal ulcers (Fahey, 2005).

Vicenin-2, quercetin and kaempferol present in AEMO leaves, hasten wound healing and reduce scar size (Muhammad *et al.*, 2013). This was observed following intake of 300 $mg.kg^{-1}$ body weight daily of the extract on some groups of rats; which was not seen in the untreated group (Rathi *et al.*, 2006). Previous studies also indicated that the paste of *Moringa oleifera* leaves could be applied externally to heal wounds (Siddhuraju and Becker, 2003).

Moringa oleifera leaves promote the production of milk in lactating women (Siddhuraju and Becker, 2003), cows and goats when ingested as feedstuff (Sánchez-Machado *et al.*, 2010). The plant is also used in the manufacturing of soaps and cosmetics (Sánchez-Machado *et al.*, 2010).

1.4.10 Safety of *Moringa oleifera*

The safety of the plant *Moringa oleifera* has been documented through research studies both on humans and research on animals without adverse outcomes (Stohs and Hartman, 2015). *Moringa* is said to be safe and non-toxic since no adverse effects were seen in the study involving administration of methanolic extracts of its seeds to rats at doses of 1000 $mg.kg^{-1}$, which is much higher than the commonly used doses in research studies (Stohs and Hartman, 2015). The commonly used doses from different extracts of the plant parts include 200 $mg.kg^{-1}$, 400 $mg.kg^{-1}$ (Bais *et al.*, 2014) and 600 $mg.kg^{-1}$ (Ahmed *et al.*, 2014).

In an investigation on toxicity conducted by Adedapo *et al.* (2009), no morbidity or mortality was observed in the acute phase following administration of aqueous extract of *Moringa oleifera* (2000 $mg.kg^{-1}$ body weight) to adult Wistar rats for 48 hours. In the sub-acute phase, a decrease in haemoglobin levels was observed in rats that received 600 $mg.kg^{-1}$ and 800 $mg.kg^{-1}$ body weight of the extract; indicating that there is tendency to develop anaemia when higher doses are used or when the period of study is prolonged (Adedapo *et al.*, 2009).

No toxic effect was reported in a study conducted by Kumari (2010) on 46 human subjects with type 2 diabetes following administration of 8 g *Moringa oleifera* leaf powder (tablet) per day for 40 days.

Moringa supplemented diet from dried crushed leaves given to rat pups at 20% and 14% of their body masses for 5 weeks had no effect on the concentrations of blood metabolites, hepatic glycogen or lipid storage and liver enzyme activities in the rats (Zvinorova *et al.*, 2015).

On the contrary, Oyagbemi *et al.* (2013) indicated that the liver and kidneys might be damaged when MEMO leaves at a dose of 400 $mg.kg^{-1}$ body weight are taken orally for eight weeks.

1.5 Fenofibrate

For this study, fenofibrate (FNF) was used as the positive control drug. FNF belongs to the fibrate class of lipid lowering agents (Nwodo *et al.*, 2014). It acts by binding to, and activating its agonist, peroxisome proliferator-activated receptor alpha (PPARα) (Mancini

et al., 2001). PPARα, a nuclear receptor, regulates enzymes and the organelles that are essential for lipid and lipoprotein metabolism (Ji *et al.*, 2005; Jeong *et al.*, 2004). This eventually leads to intracellular lipid breakdown, decrease in circulating free fatty acids and triglycerides, normalization of excess blood glucose and excess blood insulin (Jeong and Yoon, 2009). FNF also decreases total plasma cholesterol and LDL-c, and increases HDL-c concentrations (Valasek *et al.*, 2007; Winegar *et al.*, 2001).

Ji *et al.* (2005), observed that administration of FNF to Sprague Dawley rats (males) which consumed a diet high in fat for a fortnight decreased body weight gain and adiposity levels.

1.6 *Moringa oleifera* and the gastro-intestinal system

The gastro-intestinal tract (GIT) plays important roles in metabolism and growth, which include digestion and absorption of orally ingested feeds, and serving as a source of regulatory peptides and hormones like gastrin, secretin and cholecystokinin (Ajibola *et al.*, 2013). The GIT also sends satiety signals to the brain thereby helping in maintenance of energy balance (Rezvani *et al.*, 2013). The development of the GIT in rats is usually rapid during lactation and immediately after weaning both of which are stages when its morphometry can easily be affected by diet (Zvinorova *et al.*, 2015). Feeds ingested orally first come in contact with the GIT (Ajibola *et al.*, 2013). Absorption of nutrients in the gut can be affected by phytochemicals in plants when given orally thereby affecting the development of the gut and its accessory organs; this may eventually affect the growth performance of the animal (Pérez *et al.*, 2007). Thus the effect of MEMO leaves on the growth performance and the GIT of growing rats was explored in this study.

1.7 Study justification

Metabolic syndrome (MS) and its risk factors is not confined to adults, it is also observed in children and adolescents (Pandita *et al.*, 2016). For example, type 2 diabetes (a risk factor in MS) is now being reported in these younger age groups in many countries (Eckel *et al.*, 2005; Zimmet *et al.*, 2005), with nearly equal prevalence between males and females (Cirillo *et al.*, 2006). The world health organization has encouraged the exploration of phytomedicines using medicinal plants (Jaiswal *et al.*, 2009) as an adjunct to primary health care. There is however a need to scientifically

validate the efficacy of phytomedicines and in doing so, most studies have used adult male rats (Divi *et al.*, 2012) to evaluate the medicinal potential of plants. The studies also tended to focus on a single risk factor of MS, either type 2 diabetes (Erasto *et al.*, 2005), hypertension (Faizi *et al.*, 1998) or obesity (Bais *et al.*, 2014) in exploring the therapeutic potential of plants. Given the epidemiology of metabolic syndrome, it is important to design studies to investigate the medicinal potential of plants by using growing animals from both sexes and to investigate several risk factors of MS concomitantly so as to provide holistic therapeutic options.

Moringa oleifera is widely exploited for its medicinal and nutritional benefits (Sangkitikomol *et al.*, 2014). Several studies have validated its use as a treatment for obesity using adult male rats (Ahmed *et al.*, 2014; Bais *et al.*, 2014; Jain *et al.*, 2010). However, there is inadequate information on the metabolic protective effect of *Moringa oleifera* in children. This research was thus undertaken so as to add to the body of knowledge on the impact of methanolic extract of *Moringa oleifera* leaves on fructose-induced metabolic dysfunction in growing male and female Sprague Dawley rats as an experimental animal model for children at risk of developing metabolic syndrome from over-consumption of fructose.

1.8 Aim and study objectives

The study primarily aimed to explore the effects of methanolic extracts of *Moringa oleifera* leaves on fructose-induced metabolic dysfunction in growing Sprague Dawley rats.

Specifically the objectives of the research undertaken were to scientifically scrutinize the ramifications of *Moringa oleifera* on:

a. the growth performance of the rats by evaluating the rats' body mass, body mass index (BMI), waist circumference, empty carcass mass and linear growth (through-measuring the masses, lengths and Seedor indexes of their femora and tibiae)
b. the markers of high-fructose diet induced metabolic dysfunction, which are the circulating fasting metabolic substrates (glucose, triglycerides and cholesterol)
c. plasma concentration of insulin- a regulatory hormone of metabolism

d. the morphometry of the gastro-intestinal tract (GIT) and accessory organs, specifically the mass and length of the GIT and accessory organs
e. markers of visceral obesity (visceral fat pad and epididymal fat pad mass)
f. the lipid content of the liver
g. the general health profile by measuring in blood:
    - liver function enzymes particularly alanine aminotransferase (ALT) and alkaline phosphatase (ALKP)
    - plasma creatinine, blood urea nitrogen (BUN) and urea to creatinine ratio

1.9 Hypotheses

H_1: Methanolic extracts of *Moringa oleifera* leaves prevent the development of fructose-induced metabolic dysfunction in growing male and female Sprague Dawley rats.

H_0: Methanolic leaf extracts of *Moringa oleifera* donot avert the fructose-induced metabolic dysfunction from developing in growing male and female Sprague Dawley rats.

CHAPTER TWO- MATERIALS AND METHODS

2.1 Study setting

This research was undertaken in Johannesburg (South Africa) in 2015 and 2016 in the rodent unit of the Central Animal Service (CAS) and in the School of Physiology, Faculty of Health Sciences, University of the Witwatersrand.

2.2 Ethical clearance

Ethical clearance for this research as well as approval for subsequent amendments was accorded by the Animal Ethics Screening Committee (AESC) of the University of the Witwatersrand (Reference: 2015/11/51/B). The certificates are included in the appendices (appendices 1 and 2) to this dissertation.

2.3 Plant collection and preparation

Moringa oleifera leaves were collected from the Agricultural Research Council Research Farm situated in Roodeplaat, Pretoria, Republic of South Africa. Fresh leaves (15 kg) were desiccated in an oven (Salvis®, Salvis Lab, Schweiz, Switzerland) set at 40ºC (Dangarembizi *et al.*, 2014), then comminuted to fine powder with a blender (Waring Commercial Blender, HGB2WTG4, USA) (Gupta *et al.*, 2012). The powder was extracted with absolute methanol (Merck (pty) Ltd, South Africa) (100g in 400 ml) on a shaker for 24 hours (Gupta *et al.*, 2012). The mixture was then filtered through Whatmann No.1 filter paper. The filtrate was concentrated on a rotary evaporator (Buchi Rotavapor-R, Buchi LaboratoriumsTechnik AG, Schweiz, Switzerland) at 48ºC. The condensed extract was then oven-dried (Salvis®, Salvis Lab, Schweiz, Switzerland) at 40ºC (Dangarembizi *et al.*, 2014), and stored in dark tightly sealed bottles until use. The extract yield was 12%.

2.4 Animal and housing

One hundred and two (102) weaned male and female Sprague Dawley rats which were twenty one days old were housed in a well ventilated rodent room. The rodents were individualized in standard rat cages containing clean wood shavings for bedding. Each cage is 42 cm × 27 cm, made from polycarbon and covered with stainless steel mesh lid. The CAS ambient temperature in the room was set at 26 ± 2°C. The lights in the animal room were set on a 12 hour lighting cycle (illumination was turned on between 7am-7pm). The rats were allowed to acclimatize to the environmental conditions for 4 to 6 days before commencing the intervention.

2.5 Experimental design and treatment dosages

The one hundred and two rats from 11 different litters were randomly apportioned to six groups which were replicated by sex. All groups received standard rat chow *ad libitum* for the whole duration of the study. The *Moringa oleifera* extract (400 $mg.kg^{-1}$ body weight) and fenofibrate (100 $mg.kg^{-1}$ body weight) were suspended in 2 ml flavoured gelatine cubes. To account for weight variations, the concentration of the extracts and fenofibrate in the cubes were calculated to accommodate a range of 50 g body weight interval for each rat. Group I (control group, n= 19; 9 Males, 10 Females) received plain drinking water and plain gelatine cubes. Group II (n= 18; 9 Males, 9 Females) received 20% fructose solution (Mamikutty *et al.*, 2014) as drinking fluid and plain gelatine cubes. Group III (n= 17; 9 Males, 8 Females) received 20% fructose solution as drinking fluid and 400 $mg.kg^{-1}$ body weight of methanolic extract of *Moringa oleifera* (Bais *et al.*, 2014) suspended in gelatine cubes. Group IV (n= 16; 8 Males, 8 Females) received 20% fructose solution to drink and 100 $mg.kg^{-1}$ body weight of fenofibrate (Ji *et al.*, 2005) suspended in gelatine cubes. Group V (n= 16; 8 Males, 8 Females) received 400 $mg.kg^{-1}$ body weight of methanolic extract of *Moringa oleifera* in gelatine cubes and had plain drinking water. Group VI (n= 16; 8 Males, 8 Females) received 100 $mg.kg^{-1}$ body weight of fenofibrate in gelatine cubes and also had plain water to drink.

2.6 Fructose and treatment vehicle preparation

The twenty percent (20%) fructose solution was prepared based on weight by volume formula, in which 200 g of fructose was diluted in one litre of tap water (Mamikutty *et al.*, 2014). The drinking bottles containing either 20% fructose solution or plain drinking water were emptied and washed twice a week, and replaced with fresh solutions. A drop of food colourant (Robertsons Food Colouring, Libstar Operations (pty) Ltd, South Africa) was added to the stock solutions for ease of recognition of the different fluids.

Gelatine cubes were made using 8 g gelatine (Davis gelatine, Johannesburg, South Africa), 8 g brown sugar (Tongaat Hulett South Africa Ltd), 5 ml Bovril (Bovril, Uniliver, Johannesburg, South Africa) and 100 ml of boiling water [method modified from (Kamerman *et al.*, 2004)].

Fenofibrate, which served as our positive control, was dispensed at 100 $mg.kg^{-1}$ body weight. The drug is a lipid lowering agent that also possesses hypoglycaemic and

hypoinsulinaemic properties (Jeong and Yoon, 2009). Previous studies conducted on rat models have used similar dose of the drug to evaluate its hypolipidaemic properties (Larsen *et al.*, 2003; Lee *et al.*, 2002).

The rats were received in 11 batches of 8-12 rats each weekly for ease of handling and terminal sampling. The treatment period was for 10 weeks. The choice of study length was based on previous studies which used between 8 weeks (Divi *et al.*, 2012) and 12 weeks (Ahmed *et al.*, 2014) to successfully induce metabolic dysfunction with dietary fructose in rats.

2.7 Body mass measurement

Weighing of the rats with a digital scale (Snowrex Electronic Scale, Clover Scales, Johannesburg) was undertaken individually at induction, and then twice a week (Bais *et al.*, 2014) to determine body mass gain (BMG). The BMG was used to enable adjustment in the concentration of the treatments to ensure a constant dose delivery (Zvinorova *et al.*, 2015).

2.8 Terminal procedures

Following the 10 week treatment duration, the rats were deprived of food overnight for 12 hours, during which they were allowed to take plain drinking water only. Two drops of blood were taken following a pin prick to the tail vein. Fasting blood glucose and triglyceride concentrations were measured. The fasting concentration of glucose in the blood was quantified using a glucose meter (Ndong *et al.*, 2007) (Contour Plus Bayer Health Care, Diabetes Care, Isando, South Africa) according to manufacturer's instructions. A hand-held triglyceride meter (Accutrend, Roche Diagnostics, Germany) which was calibrated was used to quantify the triglycerides according to instructions of the manufacturer. The rats were then killed with sodium pentobarbitone (Centaur Laboratories, Johannesburg, South Africa) at 150 $mg.kg^{-1}$ body weight intraperitoneally (Zvinorova *et al.*, 2015). After euthanasia, BMI was determined using body weight and body length (nose-anus length) as follows (Ahmed *et al.*, 2014):

$$BMI\ (g.cm^{-2}) = Body\ weight\ (g) \div Length^2\ (cm^2)$$

Body length and waist circumference were determined using a measuring tape.

A ventral midline incision was made on the thorax and abdomen, and intra-cardiac blood was drawn into syringes via attached needles. The blood samples were then transferred into heparinized and plain tubes (BD Vacutainer, Plymouth, UK) and centrifuged (Rotofix 32A, Hettich Zentrifugen, Germany) at 3700 revolutions per minute for 15 minutes. The supernatant (plasma and serum) were then transferred into micro-tubes (Greiner bio-one Diagnostics GmbH, Austria) by pipette and kept at -20˚C for later assays. The thoracic and abdominal viscera, visceral and epididymal fat (males), and testes (males) were meticulously removed. The contents of entire gastro-intestinal tract were carefully emptied (Ndong *et al.*, 2007). The masses of these tissues were ascertained on an electronic balance (Presica 310M balance, Presica Instruments AG, CH-Dietikon, Switzerland) (Zvinorova *et al.*, 2015). The lengths of small and large intestines were determined by stretching them carefully on a cooled board and measured with a ruler (Zvinorova *et al.*, 2015). A sample of approximately two thirds of each extirpated liver was preserved at temperature of -20˚C for quantification of lipid content.

Empty carcasses following evisceration were also weighed (Snowrex Electronic Scale, Clover Scales, Johannesburg).

2.9 Linear growth

The right femur with the attached tibia were dissected away from the acetabulum, defleshed and disarticulated. An oven (Salvis®, Salvis Lab, Schweiz, Switzerland) was used to dry the bones at 40ºC for 5 days until the mass remained unchanged and then the weight established on a Presica 310M balance (Zvinorova *et al.*, 2015) to determine their dry masses. The lengths of the bones were measured using KTV 150 mm Digital Caliper (MAJOR TECH (pty) LTD, Elandsfontein, South Africa).

The bones' Seedor indexes were computed with a formula (Almeida *et al.*, 2008):

Seedor index= Bone mass (mg) ÷ Bone length (mm)

A Fujifilm digital X-ray system (Industrial X-ray film FR; Fuji Photo Film Co., Ltd, Tokyo, Japan) was used for bone radiographs to enable subjective assessment of the bones' densities. The bones were placed on a photographic plate set 100 cm away from the source of the X-ray light. The source of the light was set at 52 kVp and 0.80 mA with a ten second exposure time.

2.10 Hepatic lipid content determination

This was carried out at the Agricultural Research Council (Irene Analytical Services Laboratory) by solvent extraction. The Tecator Soxtec method (Official Methods of Analysis of Analytical Chemists, 2005) was used. The liver specimens were milled after having been freeze-dried. A gram (1 g) was put into a pre-weighed extraction thimble. The thimble was plugged with cotton wool (fat-free) before being put on a thimble holder. Before being placed onto the heating pads, petroleum ether was added to the extraction cups. Four stages were employed for the extraction process: boiling for 30 minutes, rinsing for 30 minutes, recovery of the solvent for 10 minutes then finally dessication for 30 minutes at 90 ± 5˚C. The thimbles used for extraction were cooled in a dessicator. The extracted lipid from the hepatic samples was calculated using the formula:

% lipid = [(mass of thimble with fat – mass of thimble) ÷ (mass of sample)] x 100

The samples were analyzed in triplicate.

2.11 Determination of clinical biochemical parameters

Plasma concentrations of cholesterol, blood urea nitrogen (BUN), creatinine, alkaline phosphatase (ALKP) and alanine aminotransferase (ALT) were determined using a calibrated VetTest analyzer (IDEXX VetTest® Clinical Chemistry Analyzer, IDEXX Laboratories Inc., USA) as instructed by the manufacturer. The ALKP and ALT were measured in units of activity.

Fasting serum insulin concentration ($ng.ml^{-1}$) was ascertained using an Enzyme Linked Immuno-Sorbent Assay (ELISA) kit (Elabscience Biotechnology Co., Ltd) (Crescenzo *et al.*, 2014).

The insulin concentration ($ng.ml^{-1}$) was then expressed as ($\mu U.ml^{-1}$) using the formula: 1 $ng.ml^{-1}$ is equivalent to 0.02 $\mu U.ml^{-1}$.

The insulin resistance index was then computed according to the Homeostasis Model of Assessment (HOMA-IR) using the following formula (Divi *et al.*, 2012):

"HOMA-IR = fasting insulin ($\mu U.ml^{-1}$) × fasting glucose ($mmol.l^{-1}$) ÷ 22.5"

2.12 Data analysis

Data analysis was done using GraphPad Prism 5.0v for windows (GraphPad Software, Inc. CA). Data were expressed as mean ± standard deviation. A one way or two-way analysis of variance (ANOVA) was used to analyze the data subject to the variables. A Bonferroni post hoc test was also done for comparison of the means. Statistical significance was considered at $P \leq 0.05$.

CHAPTER THREE- RESULTS

3.1 Morbidity and mortality of the animals

There were no clinical morbidities that required any animal to be removed from the study and there were no mortalities recorded.

3.2 Effects of methanolic extract of *Moringa oleifera* leaves on growth performance

3.2.1 Body mass

Figures 3.1A and 3.1B show the absolute initial and terminal body masses of the study rats. There were insignificant differences ($P>0.05$) in the initial body masses of the rats across all treatment groups. After 10 weeks of treatment, all animals had a significant increase ($p<0.0001$) in body mass.

The male rats that received combined fructose and fenofibrate had significantly lower body masses at termination compared to the negative controls ($p<0.0001$) as well as those that received *Moringa* alone ($p<0.0001$) and those that received fructose alone ($P<0.05$).

In females, there was an insignificant difference observed ($p>0.05$) in the terminal body mass between the rats across the groups.

A comparative analysis showed that the male rats had significantly higher ($P<0.0001$) terminal body masses than female rats across all treatments.

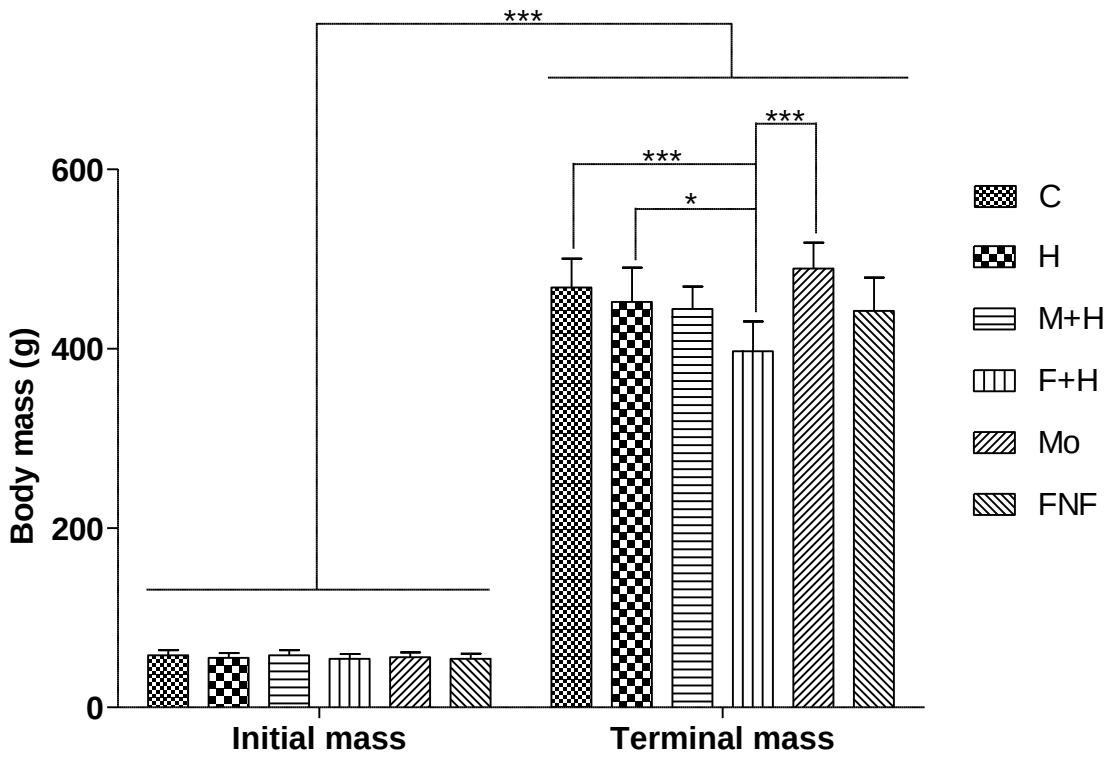

Figure 3.1A: Effects of methanolic extract of *Moringa oleifera* leaves on body mass of male Sprague Dawley rats.

Data was expressed as mean ± standard deviation**. *****= significantly different at P<0.0001; *= significantly different at P<0.05; C= negative control (plain drinking water and plain gelatine cubes) (n= 19; 9 males, 10 females); H= 20% fructose solution and plain gelatine cubes(n= 18; 9 males, 9 females); M+H= 400 mg.kg^{-1} of methanolic extract of *Moringa oleifera* leaves in gelatine cubes and 20% fructose solution (n= 17; 9 males, 8 females); F+H= 100 mg.kg^{-1} of fenofibrate in gelatine cubes and 20% fructose solution (n= 16; 8 males, 8 females); Mo= 400 mg.kg^{-1} of methanolic extract of *Moringa oleifera* leaves in gelatine cubes and plain drinking water (n= 16; 8 males, 8 females); FNF= 100 mg.kg^{-1} of fenofibrate in gelatine cubes and plain drinking water (n= 16; 8 males, 8 females).

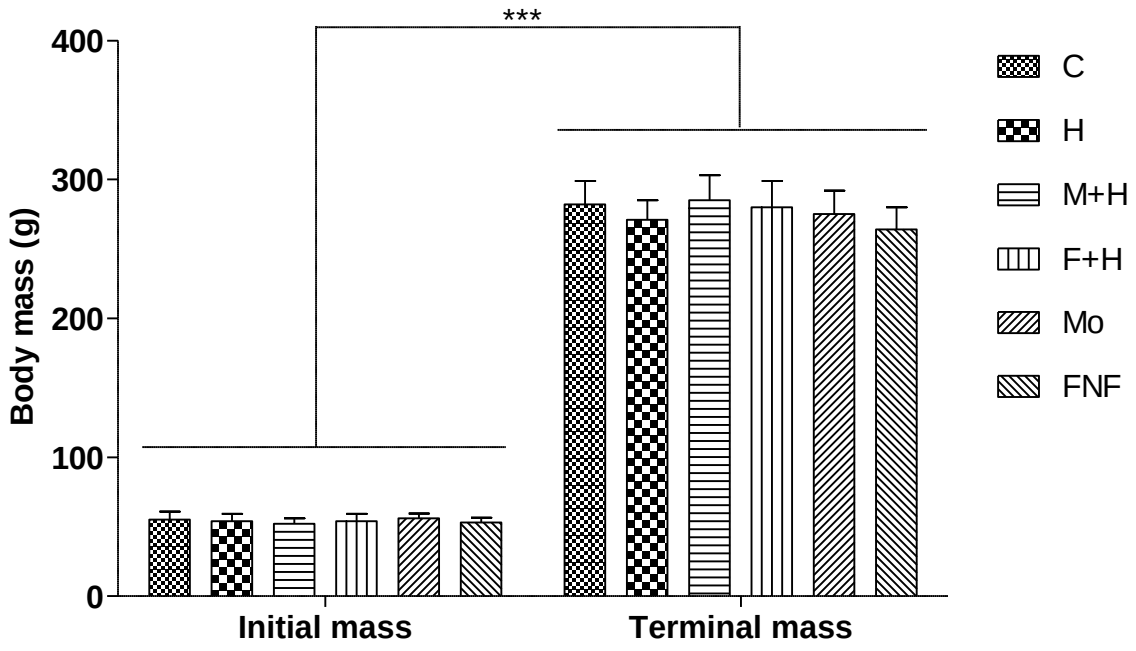

Figure 3.1B: Effects of methanolic extract of *Moringa oleifera* leaves on body mass of female Sprague Dawley rats.

Data was expressed as mean ± standard deviation**. *****= significantly different at P<0.0001; C= negative control (plain drinking water and plain gelatine cubes) (n= 19; 9 males, 10 females); H= 20% fructose solution and plain gelatine cubes (n= 18; 9 males, 9 females); M+H= 400 $mg.kg^{-1}$ of methanolic extract of *Moringa oleifera* leaves in gelatine cubes and 20% fructose solution (n= 17; 9 males, 8 females); F+H= 100 $mg.kg^{-1}$ of fenofibrate in gelatine cubes and 20% fructose solution (n= 16; 8 males, 8 females); Mo= 400 $mg.kg^{-1}$ of methanolic extract of *Moringa oleifera* leaves in gelatine cubes and plain drinking water (n= 16; 8 males, 8 females); FNF= 100 $mg.kg^{-1}$ of fenofibrate in gelatine cubes and plain drinking water (n= 16; 8 males, 8 females).

3.2.2 Body mass gain

Figures 3.2A and 3.2B show the effects of the extract on body mass gain (percentage body mass difference from initiation to termination) of the study rats. Male rats on combined fructose and fenofibrate, and those on combined fructose and *Moringa* had significantly lower ($P \leq 0.05$) body mass gain in comparison to the rats that received *Moringa* alone. No significant difference was observed among other groups ($P > 0.05$).

In females, the body mass gain among the different treatment groups was not significantly different ($P > 0.05$).

A comparative analysis showed that there was a significantly higher ($P < 0.0001$) body mass gain in male rats in comparison to females in all treatment groups.

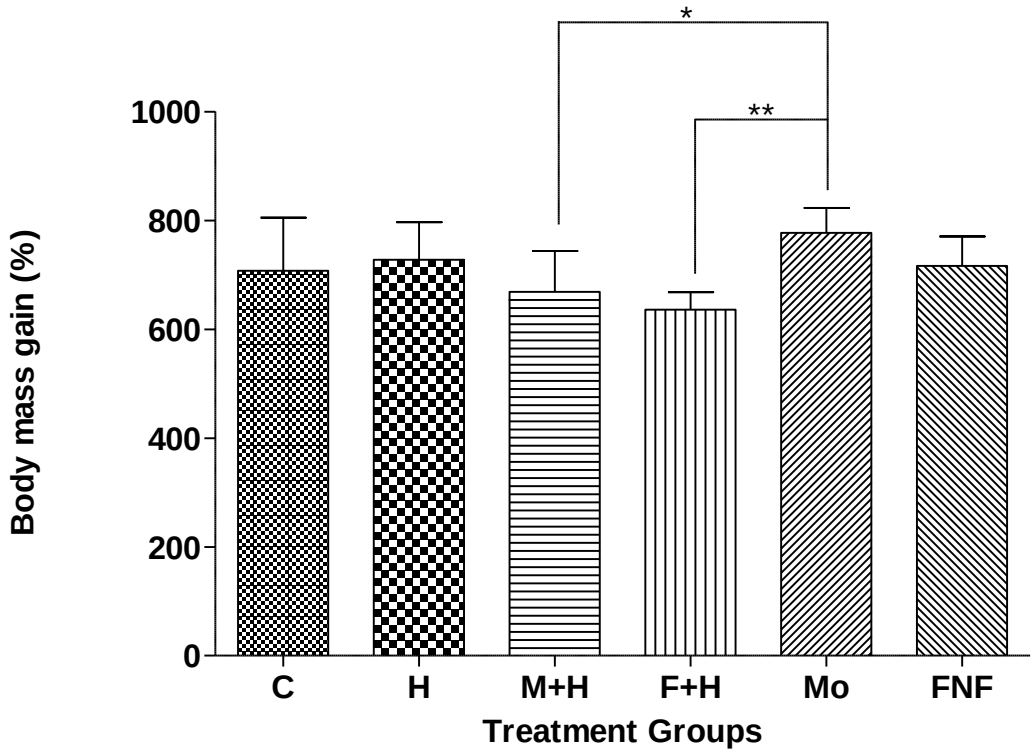

Figure 3.2A: Effects of methanolic extract of *Moringa oleifera* leaves on body mass gain of male Sprague Dawley rats.

Data was expressed as mean ± standard deviation**.** *= significantly different at P<0.05; **= significantly different at P<0.01; C= negative control (plain drinking water and plain gelatine cubes) (n= 19; 9 males, 10 females); H= 20% fructose solution and plain gelatine cubes (n= 18; 9 males, 9 females); M+H= 400 $mg.kg^{-1}$ of methanolic extract of *Moringa oleifera* leaves in gelatine cubes and 20% fructose solution (n= 17; 9 males, 8 females); F+H= 100 $mg.kg^{-1}$ of fenofibrate in gelatine cubes and 20% fructose solution (n= 16; 8 males, 8 females); Mo= 400 $mg.kg^{-1}$ of methanolic extract of *Moringa oleifera* leaves in gelatine cubes and plain drinking water (n= 16; 8 males, 8 females); FNF= 100 $mg.kg^{-1}$ of fenofibrate in gelatine cubes and plain drinking water (n= 16; 8 males, 8 females).

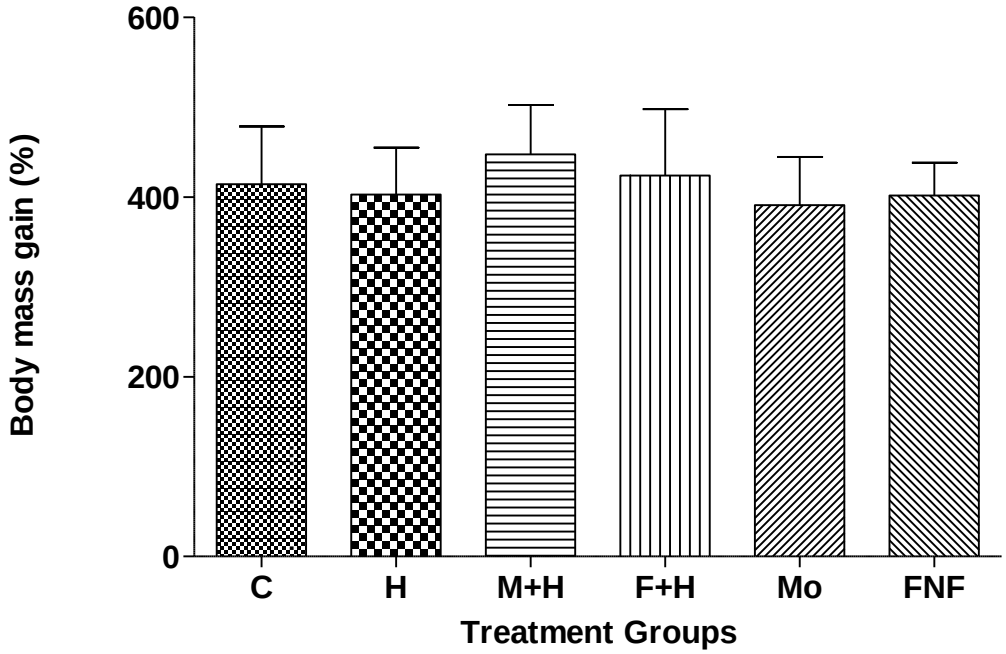

Figure 3.2B: Effects of methanolic extract of *Moringa oleifera* leaves on body mass gain of female Sprague Dawley rats.

Data was expressed as mean ± standard deviation**.** The differences were insignificant across different treatment groups (P>0.05); C= negative control (plain drinking water and plain gelatine cubes) (n= 19; 9 males, 10 females); H= 20% fructose solution and plain gelatine cubes (n= 18; 9 males, 9 females); M+H= 400 $mg.kg^{-1}$ of methanolic extract of *Moringa oleifera* leaves in gelatine cubes and 20% fructose solution (n= 17; 9 males, 8 females); F+H= 100 $mg.kg^{-1}$ of fenofibrate in gelatine cubes and 20% fructose solution (n= 16; 8 males, 8 females); Mo= 400 $mg.kg^{-1}$ of methanolic extract of *Moringa oleifera* leaves in gelatine cubes and plain drinking water (n= 16; 8 males, 8 females); FNF= 100 $mg.kg^{-1}$ of fenofibrate in gelatine cubes and plain drinking water (n= 16; 8 males, 8 females).

3.2.3 Body mass index (BMI), waist circumference (WC) and empty carcass mass

Table 3.1 shows the body mass indexes, waist circumferences and empty carcass masses of the rats across different treatment groups. In males, rats on combined fructose and fenofibrate had significantly lower ($p<0.05$) BMI compared to rats on *Moringa* alone. No significant difference was observed ($p>0.05$) in the BMI among other treatment groups. Rats on fenofibrate alone had significantly lower ($p<0.05$) WC compared to those on either *Moringa* alone or fructose alone. No significant difference was observed ($p>0.05$) in WC of other treatment groups. Rats on combined fructose and fenofibrate had significantly lower ($P<0.01$) empty carcass masses compared to those on fructose alone. Rats on combined fructose and fenofibrate had significantly lower ($P<0.0001$) empty carcass masses compared to negative control group and those on *Moringa* alone. Rats receiving fenofibrate alone had significantly lower ($P<0.01$) empty carcass masses compared to those on *Moringa* alone. Rats on combined fructose and *Moringa* had significantly lower ($P<0.05$) empty carcass masses compared to those on *Moringa* alone.

In females, no significant difference was observed ($p>0.05$) in the BMI, WC and empty carcass masses of the rats across different treatment groups.

Male rats had significantly higher ($P<0.0001$) BMI, WC and empty carcass masses compared to female rats.

Table 3.1: Effects of methanolic extract of *Moringa oleifera* leaves on body mass index, waist circumference and empty carcass mass of the study rats

Parameter	Sex	C	H	M+H	F+H	Mo	FNF
BMI ($g.cm^{-2}$)	Male	0.69 ± 0.04^{ab}	0.69 ± 0.04^{ab}	0.71 ± 0.05^{ab}	0.66 ± 0.04^{a}	0.73 ± 0.04^{b}	0.69 ± 0.05^{ab}
	Female	$0.59\pm0.03^{a\#}$	$0.58\pm0.03^{a\#}$	$0.59\pm0.03^{a\#}$	$0.60\pm0.03^{a\#}$	$0.59\pm0.05^{a\#}$	$0.56\pm0.03^{a\#}$
WC (cm)	Male	20.00 ± 0.77^{ab}	20.00 ± 1.60^{a}	20.00 ± 1.00^{ab}	19.00 ± 0.79^{ab}	20.00 ± 0.65^{a}	19.00 ± 0.72^{b}
	Female	$16.00\pm0.63^{a\#}$	$17.00\pm0.94^{a\#}$	$17.00\pm1.40^{a\#}$	$16.00\pm0.62^{a\#}$	$17.00\pm1.50^{a\#}$	$16.00\pm0.52^{a\#}$
Empty carcass (g)	Male	368.00 ± 26.00^{ab}	361.00 ± 33.00^{ab}	336.00 ± 35.00^{abc}	298.00 ± 29.00^{ac}	385.00 ± 23.00^{ab}	326.00 ± 29.00^{abc}
	Female	$225.00\pm29.00^{a\#}$	$207.00\pm11.00^{a\#}$	$219.00\pm13.00^{a\#}$	$212.00\pm12.00^{a\#}$	$212.00\pm11.00^{a\#}$	$202.00\pm12.00^{a\#}$

Data was expressed as mean ± standard deviation. ^{abc}= within row means with different letters in superscript significantly different at P≤0.05; $^{\#}$= female rats had significantly lower BMI, WC and empty carcass masses than male rats (P<0.0001); C= negative control (plain drinking water and plain gelatine cubes) (n= 19; 9 males, 10 females); H= 20% fructose solution and plain gelatine cubes (n= 18; 9 males, 9 females); M+H= 400 $mg.kg^{-1}$ of methanolic extract of *Moringa oleifera* leaves in gelatine cubes and 20% fructose solution (n= 17; 9 males, 8 females); F+H= 100 $mg.kg^{-1}$ of fenofibrate in gelatine cubes and 20% fructose solution (n= 16; 8 males, 8 females); Mo= 400 $mg.kg^{-1}$ of methanolic extract of *Moringa oleifera* leaves in gelatine cubes and plain drinking water (n= 16; 8 males, 8 females); FNF= 100 $mg.kg^{-1}$ of fenofibrate in gelatine cubes and plain drinking water (n= 16; 8 males, 8 females); BMI= body mass index; WC= waist circumference.

3.2.4 Linear growth

Table 3.2 shows the morphometric measurements (mass, length and Seedor indexes) of the femurs and tibias of the rats across the different treatment groups. In males, rats that were given combined fructose and fenofibrate had significantly lower ($P<0.05$) tibial masses, lengths and Seedor indexes compared to those that had *Moringa* alone. Rats given combined fructose and fenofibrate had significantly lower tibial lengths ($P<0.01$) compared to the negative control group. For the measurements on the femur, rats that were given combined fructose and fenofibrate had significantly lower ($P<0.05$) femoral masses and lengths compared to those on *Moringa* alone. Rats on combined fructose and fenofibrate had significantly lower ($P<0.0001$) femoral lengths compared to the negative control group. The femoral Seedor indexes of male rats across the intervention groups were not significantly different ($P>0.05$).

In females, rats that received fructose alone had significantly lower tibial length ($P<0.05$) in comparison to the negative control group. The masses and Seedor indexes of the rats' tibiae across the groups were similar ($P>0.05$). No significant difference was observed ($P>0.05$) in the masses, lengths and Seedor indexes of female femora across the treatment groups.

Generally, females had significantly lighter masses, shorter lengths and lesser Seedor indexes of tibiae and femora than male rats across different treatment groups ($P<0.0001$).

Representative radiographs of the long bones (femora and tibiae) of the study rats are respectively shown in Figures 3.3A and 3.3B. A subjective assessment of the images does not show any differences in the density of the long bones from male and female rats in the different treatment groups.

Table 3.2: Effects of methanolic extract of *Moringa oleifera* leaves on masses, lengths and Seedor indexes of rats' femora and tibiae

Parameter	Sex	C	H	M+H	F+H	Mo	FNF
Tibial mass (mg)	Male	506.00±50.37^{ab}	483.40±31.06^{ab}	468.20±26.93^{ab}	451.80±36.88^{a}	517.60±30.93^{b}	476.00±37.22^{ab}
	Female	376.00±19.00$^{a\#}$	364.00±22.00$^{a\#}$	364.00±11.00$^{a\#}$	372.00±27.00$^{a\#}$	368.00±15.00$^{a\#}$	352.00±15.00$^{a\#}$
Tibial length (mm)	Male	41.00±0.96^{a}	40.00±0.88^{ab}	40.00±0.63^{ab}	39.00±1.30^{b}	41.00±0.51^{a}	40.00±0.66^{ab}
	Female	37.00±0.26$^{a\#}$	36.00±0.42$^{b\#}$	37.00±0.78$^{ab\#}$	36.00±0.50$^{ab\#}$	36.00±0.55$^{ab\#}$	36.00±0.58$^{ab\#}$
Seedor index	Male	12.00±1.00^{ab}	12.00±0.55^{ab}	12.00±0.62^{ab}	12.00±0.60^{a}	13.00±0.78^{b}	12.00±0.78^{ab}
	Female	10.00±0.49$^{a\#}$	10.00±0.53$^{a\#}$	9.90±0.22$^{a\#}$	10.00±0.62$^{a\#}$	10.00±0.46$^{a\#}$	9.70±0.29$^{a\#}$
Femoral mass (mg)	Male	702.00±86.00^{ab}	667.00±48.00^{ab}	657.00±54.00^{ab}	629.00±36.00^{a}	728.00±49.00^{b}	671.00±46.00^{ab}
	Female	548.00±42.00$^{a\#}$	521.00±23.00$^{a\#}$	528.00±36.00$^{a\#}$	548.00±45.00$^{a\#}$	542.00±27.00$^{a\#}$	517.00±14.00$^{a\#}$
Femoral length (mm)	Male	37.00±1.40^{a}	36.00±0.82^{ab}	36.00±1.00^{ab}	35.00±0.81^{b}	37.00±0.95^{a}	36.00±0.85^{ab}
	Female	33.00±0.98$^{a\#}$	32.00±0.79$^{a\#}$	33.00±1.50$^{a\#}$	33.00±0.96$^{a\#}$	33.00±0.89$^{a\#}$	32.00±0.72$^{a\#}$
Seedor index	Male	19.00±1.80^{a}	19.00±1.00^{a}	18.00±1.30^{a}	18.00±0.78^{a}	20.00±0.94^{a}	19.00±1.40^{a}
	Female	16.00±0.87$^{a\#}$	16.00±0.79$^{a\#}$	16.00±0.56$^{a\#}$	17.00±1.00$^{a\#}$	16.00±0.80$^{a\#}$	16.00±0.51$^{a\#}$

Data was expressed as mean ± standard deviation. ^{ab}= within row means with different letters in superscript significantly different at P≤0.05; $^{\#}$= female rats had significantly less heavier, shorter and less denser femora and tibiae than male rats (P<0.0001); C= negative control (plain drinking water and plain gelatine cubes) (n= 19; 9 males, 10 females); H= 20% fructose solution and plain gelatine cubes (n= 18; 9 males, 9 females); M+H= 400 mg.kg^{-1} of methanolic extract of *Moringa oleifera* leaves in gelatine cubes and 20% fructose solution (n= 17; 9 males, 8 females); F+H= 100 mg.kg^{-1} of fenofibrate in gelatine

cubes and 20% fructose solution (n= 16; 8 males, 8 females); Mo= 400 mg.kg^{-1} of methanolic extract of *Moringa oleifera* leaves in gelatine cubes and plain drinking water (n= 16; 8 males, 8 females); FNF= 100 mg.kg^{-1} of fenofibrate in gelatine cubes and plain drinking water (n= 16; 8 males, 8 females).

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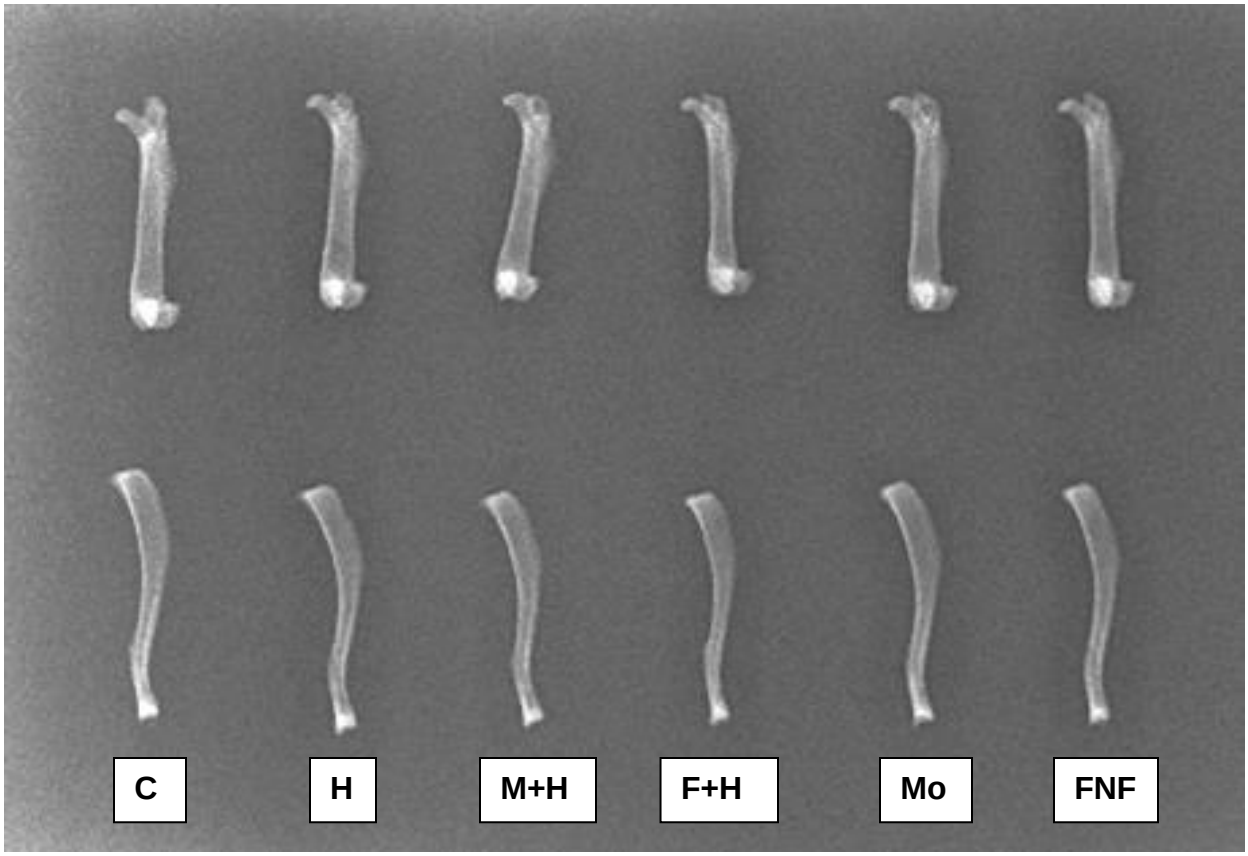

Figure 3.3A: Radiograph images of femora and tibiae of male Sprague Dawley rats. The top row shows radiographs of the femora and the bottom row shows the tibiae of representative rats from different treatment groups. C= negative control (plain drinking water and plain gelatine cubes); H= 20% fructose solution and plain gelatine cubes; M+H= 400 $mg.kg^{-1}$ of methanolic extract of *Moringa oleifera* leaves in gelatine cubes and 20% fructose solution; F+H= 100 $mg.kg^{-1}$ of fenofibrate in gelatine cubes and 20% fructose solution; Mo= 400 $mg.kg^{-1}$ of methanolic extract of *Moringa oleifera* in gelatine cubes and plain drinking water; FNF= 100 $mg.kg^{-1}$ of fenofibrate in gelatine cubes and plain drinking water.

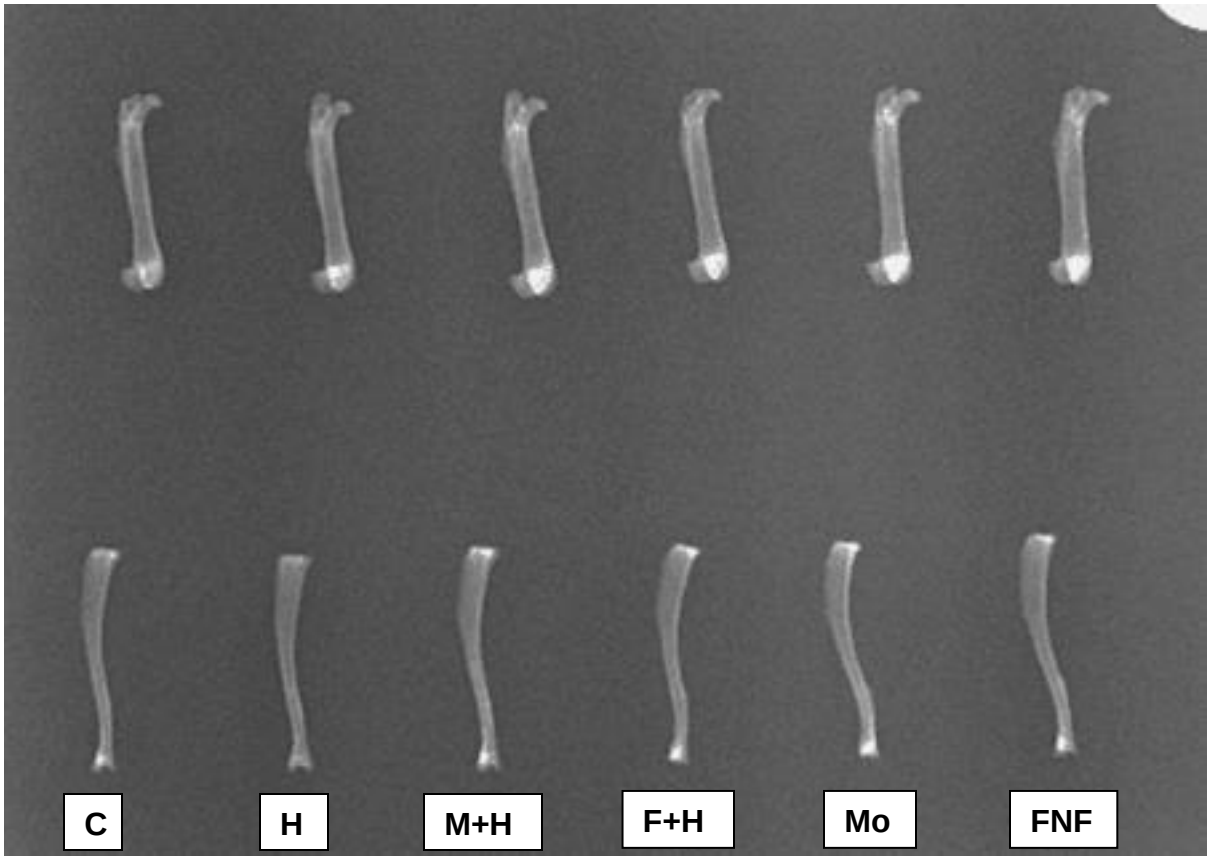

Figure 3.3B: Radiograph images of femora and tibiae of female Sprague Dawley rats. The top row shows radiographs of the femora while the bottom row shows the tibiae of representative rats from different treatment groups. C= negative control (plain drinking water and plain gelatine cubes); H= 20% fructose solution and plain gelatine cubes; M+H= 400 $mg.kg^{-1}$ of methanolic extract of *Moringa oleifera* in gelatine cubes and 20% fructose solution; F+H= 100 $mg.kg^{-1}$ of fenofibrate in gelatine cubes and 20% fructose solution; Mo= 400 $mg.kg^{-1}$ of methanolic extract of *Moringa oleifera* in gelatine cubes and plain drinking water; FNF= 100 $mg.kg^{-1}$ of fenofibrate in gelatine cubes and plain drinking water.

3.3 Effects of methanolic extract of *Moringa oleifera* leaves on the development of metabolic dysfunction

3.3.1 Glucose, triglyceride and cholesterol concentrations

Table 3.3 shows the blood glucose concentration after fasting (FBG), plasma triglycerides and cholesterol concentrations of the female and male Sprague Dawleys. In males, rats receiving fructose alone or combined with *Moringa* had lower ($P<0.0001$) FBG concentration in comparison to those receiving fenofibrate alone or combined with fructose. Rats on combined fructose and fenofibrate had higher ($P<0.01$) FBG in comparison to negative controls and those on *Moringa* alone. No significant difference was observed ($P>0.05$) in triglycerides and cholesterol concentrations among all the treatment groups.

In females, rats that had fenofibrate alone had significantly higher ($P<0.0001$) FBG versus those on combined *Moringa* and fructose and also when compared to the negative controls ($P<0.05$). Those on combined fructose and *Moringa* had significantly lower ($P<0.05$) FBG compared to those on combined fructose and fenofibrate. Fructose intake significantly increased ($P<0.05$) the plasma triglyceride concentration such that rats on fructose alone had significantly higher triglycerides concentration compared to those on negative controls on normal feed ($P<0.01$), *Moringa* alone ($P<0.0001$) and fenofibrate alone ($P<0.0001$). Treatment with *Moringa* leaf extracts and fenofibrate did reduce the fructose induced hypertriglyceridaemia. Rats on either *Moringa* alone or fenofibrate alone had similar triglyceride concentrations to the negative control. Although fructose intake did not result in a significant elevation ($P>0.05$) in plasma cholesterol, rats on fenofibrate alone had lower ($P<0.05$) cholesterol concentrations compared to those on fructose alone or negative control group.

A comparison of the sexes showed that male rats had higher ($P<0.01$) FBG, but lower ($P<0.05$) triglycerides and cholesterol concentrations compared to their female counterparts in similar treatment groups.

Table 3.3: Effects of methanolic extract of *Moringa oleifera* leaves on fasting blood glucose, triglycerides and cholesterol concentrations

Parameter	Sex	C	H	M+H	F+H	Mo	FNF
Glucose ($mmol.l^{-1}$)	Male	4.40 ± 0.34^{ac}	4.00 ± 0.42^{a}	4.10 ± 0.34^{a}	5.00 ± 0.26^{b}	4.40 ± 0.16^{ac}	4.80 ± 0.23^{bc}
	Female	$4.10\pm0.26^{ab\beta}$	$4.20\pm0.38^{ab\beta}$	$3.90\pm0.31^{b\beta}$	$4.40\pm0.40^{ac\beta}$	$4.10\pm0.34^{ab\beta}$	$4.60\pm0.23^{ac\beta}$
Triglycerides ($mmol.l^{-1}$)	Male	1.40 ± 0.35^{a}	2.10 ± 0.28^{a}	1.70 ± 0.51^{a}	2.00 ± 1.00^{a}	1.50 ± 0.37^{a}	1.70 ± 0.27^{a}
	Female	$1.70\pm0.30^{a\delta}$	$2.30\pm0.43^{b\delta}$	$2.20\pm0.38^{b\delta}$	$2.40\pm0.25^{b\delta}$	$1.50\pm0.22^{a\delta}$	$1.50\pm0.16^{a\delta}$
Cholesterol ($mg.dl^{-1}$)	Male	59.00 ± 12.00^{a}	57.00 ± 5.40^{a}	50.00 ± 15.00^{a}	55.00 ± 26.00^{a}	48.00 ± 21.00^{a}	59.00 ± 15.00^{a}
	Female	$77.00\pm10.00^{a\delta}$	$78.00\pm12.00^{a\delta}$	$73.00\pm10.00^{ab\delta}$	$75.00\pm12.00^{ab\delta}$	$70.00\pm9.50^{ab\delta}$	$60.00\pm12.00^{b\delta}$

Data was expressed as mean ± standard deviation. ^{abc}= within row means with different letters in superscripts significantly different at P≤0.05; $^{\beta}$= female rats had significantly lower fasting blood glucose levels than male rats (P<0.01); $^{\delta}$= female rats had significantly higher triglycerides and cholesterol concentrations than male rats (P<0.05); C= negative control (plain drinking water and plain gelatine cubes) (n= 19; 9 males, 10 females); H= 20% fructose solution and plain gelatine cubes (n= 18; 9 males, 9 females); M+H= 400 $mg.kg^{-1}$ of methanolic extract of *Moringa oleifera* leaves in gelatine cubes and 20% fructose solution (n= 17; 9 males, 8 females); F+H= 100 $mg.kg^{-1}$ of fenofibrate in gelatine cubes and 20% fructose solution (n= 16; 8 males, 8 females); Mo= 400 $mg.kg^{-1}$ of methanolic extract of *Moringa oleifera* leaves in gelatine cubes and plain drinking water (n= 16; 8 males, 8 females); FNF= 100 $mg.kg^{-1}$ of fenofibrate in gelatine cubes and plain drinking water (n= 16; 8 males, 8 females).

3.3.2 Serum insulin and the HOMA-IR index

Table 3.4 shows the concentrations of insulin in serum and the HOMA-IR indexes of study rats of both sexes. The insulin concentrations in serum and computed HOMA-IR indexes across all treatment groups were not significantly different in both sexes ($P>0.05$).

However, the males had significantly higher insulin concentrations in serum and HOMA-IR indexes ($P<0.0001$) compared to female rats in corresponding groups.

Table 3.4: Effect of methanolic extract of *Moringa oleifera* leaves on serum insulin concentration and HOMA-IR index

Parameter	Sex	C	H	M+H	F+H	Mo	FNF
Insulin ($ng.ml^{-1}$)	Male	44.00±33.00	62.00±36.00	41.00±20.00	35.00±33.00	43.00±37.00	20.00±12.00
	Female	23.00±9.90#	23.00±19.00#	17.00±9.50#	22.00±12.00#	24.00±20.00#	19.00±12.00#
HOMA-IR Index	Male	8.90±7.10	11.00±7.20	7.40±3.50	7.80±7.50	8.50±7.30	4.10±2.60
	Female	4.20±1.60#	4.40±3.60#	2.80±1.60#	4.40±2.50#	4.70±4.30#	3.90±2.40#

Data was expressed as mean ± standard deviation. There was no significant difference across different treatment groups ($P>0.05$); #= female rats had significantly lower Insulin concentrations and HOMA-IR indexes than male rats ($P<0.0001$); C= negative control (plain drinking water and plain gelatine cubes) (n= 19; 9 males, 10 females); H= 20% fructose solution and plain gelatine cubes (n= 18; 9 males, 9 females); M+H= 400 $mg.kg^{-1}$ of methanolic extract of *Moringa oleifera* leaves in gelatine cubes and 20% fructose solution (n= 17; 9 males, 8 females); F+H= 100 $mg.kg^{-1}$ of fenofibrate in gelatine cubes and 20% fructose solution (n= 16; 8 males, 8 females); Mo= 400 $mg.kg^{-1}$ of methanolic extract of *Moringa oleifera* leaves in gelatine cubes and plain drinking water (n= 16; 8 males, 8 females); FNF= 100 $mg.kg^{-1}$ of fenofibrate in gelatine cubes and plain drinking water (n= 16; 8 males, 8 females); HOMA-IR= Homeostatic model of insulin resistance.

3.3.3 Visceral fat and epididymal fat pad masses

The masses of fat pads (abdominal visceral) and epididymal (males only) of the rats following the 10 week treatment period are shown in Table 3.5. In males, rats receiving fructose alone and those that received *Moringa* alone had a significant increase ($P<0.05$) in both absolute visceral fat and visceral fat relative to tibial length compared to rats that had combined fructose and fenofibrate. The differences observed in the absolute mass of epididymal fat between all the groups were statistically insignificant ($P>0.05$). However, when expressed relative to tibial length, rats that received fenofibrate alone or combined with fructose had a significantly lower ($P<0.05$) amount of epididymal fat relative to tibial length compared to all the other groups.

In female rats, those that received fenofibrate alone had a significantly lower ($P<0.01$) absolute and relative (to tibial length) visceral fat mass compared to those on combined fructose and *Moringa*.

In comparison, female rats had significantly higher absolute visceral fat ($P<0.05$) and that relative to tibial length ($P<0.0001$) than their male counterparts.

Table 3.5: Effects of methanolic extract of *Moringa oleifera* leaves on masses of visceral fat and epididymal fat pads

Fat pad mass	Sex	C	H	M+H	F+H	Mo	FNF
Visceral (g)	Male	13.00 ± 3.40^{ab}	15.00 ± 2.60^{a}	14.00 ± 2.10^{ab}	9.60 ± 2.40^{b}	14.00 ± 3.60^{a}	11.00 ± 1.70^{ab}
	Female	$14.00\pm2.10^{ab\#}$	$15.00\pm2.60^{ab\#}$	$17.00\pm3.40^{a\#}$	$13.00\pm3.70^{ab\#}$	$14.00\pm2.00^{ab\#}$	$11.00\pm3.00^{b\#}$
Visceral (grTL)	Male	0.32 ± 0.08^{ab}	0.36 ± 0.06^{a}	0.34 ± 0.05^{ab}	0.24 ± 0.06^{b}	0.35 ± 0.09^{a}	0.29 ± 0.04^{ab}
	Female	$0.38\pm0.06^{ab\#}$	$0.42\pm0.07^{ab\#}$	$0.46\pm0.09^{a\#}$	$0.34\pm0.10^{ab\#}$	$0.39\pm0.06^{ab\#}$	$0.31\pm0.09^{b\#}$
Epididymal (g)	Male	4.10 ± 1.10^{a}	4.10 ± 0.77^{a}	4.80 ± 3.20^{a}	2.70 ± 0.60^{a}	4.10 ± 1.00^{a}	2.80 ± 0.32^{a}
Epididymal (grTL)	Male	0.10 ± 0.03^{a}	0.10 ± 0.02^{a}	0.09 ± 0.01^{ab}	0.07 ± 0.02^{b}	0.10 ± 0.03^{a}	0.07 ± 0.01^{b}

Data was expressed as mean ± standard deviation. ^{ab}= within row means with different letters in superscript significantly different at P≤0.05; $^{\#}$= female rats had significantly higher absolute visceral fat and that relative to tibial length than male rats (P<0.0001); C= negative control (plain drinking water and plain gelatine cubes) (n= 19; 9 males, 10 females); H= 20% fructose solution and plain gelatine cubes (n= 18; 9 males, 9 females); M+H= 400 $mg.kg^{-1}$ of methanolic extract of *Moringa oleifera* leaves in gelatine cubes and 20% fructose solution (n= 17; 9 males, 8 females); F+H= 100 $mg.kg^{-1}$ of fenofibrate in gelatine cubes and 20% fructose solution (n= 16; 8 males, 8 females); Mo= 400 $mg.kg^{-1}$ of methanolic extract of *Moringa oleifera* leaves in gelatine cubes and plain drinking water (n= 16; 8 males, 8 females); FNF= 100 $mg.kg^{-1}$ of fenofibrate in gelatine cubes and plain drinking water (n= 16; 8 males, 8 females); grTL= mass relative to tibial length.

3.3.4 Hepatic lipids storage

Figures 3.4A and 3.4B show the impact of methanolic leaf extracts of *Moringa oleifera* on hepatic lipid storage of the study rats. In males, rats intake of fructose alone resulted in significantly more liver lipid accumulation in comparison to the negative controls and those on *Moringa* alone ($P<0.0001$). Treatment with fenofibrate significantly lowered ($P<0.05$) content of liver lipid compared to those on fructose alone (FNF vs H; $P<0.05$) and all the other groups in the study ($P<0.05$). However *Moringa* was unable to reduce the fructose induced hepatic lipid accumulation (H vs M+H; $P>0.05$).

In females, rats on fructose alone had significantly more ($P<0.05$) liver lipid content in comparison to the negative controls and the rats on *Moringa* alone ($P<0.05$). Unlike in males, in the females *Moringa* significantly reduced the fructose induced accumulation of hepatic lipids (M+H vs H; $p<0.05$).Treatment with fenofibrate significantly reduced fructose-induced lipid accumulation (F+H vs H) to a significantly greater extent than *Moringa* (F+H vs M+H; $P<0.01$).Female rats on fenofibrate alone had significantly lower liver lipid content compared to all other groups ($P<0.001$) except those on *Moringa* alone. Rats on *Moringa* alone also had significantly lower lipid content compared to all other groups ($P<0.05$).

The comparative analysis for sex differences showed that the amount of lipids in the livers of the male and female rats were similar ($P>0.05$) in corresponding treatment groups.

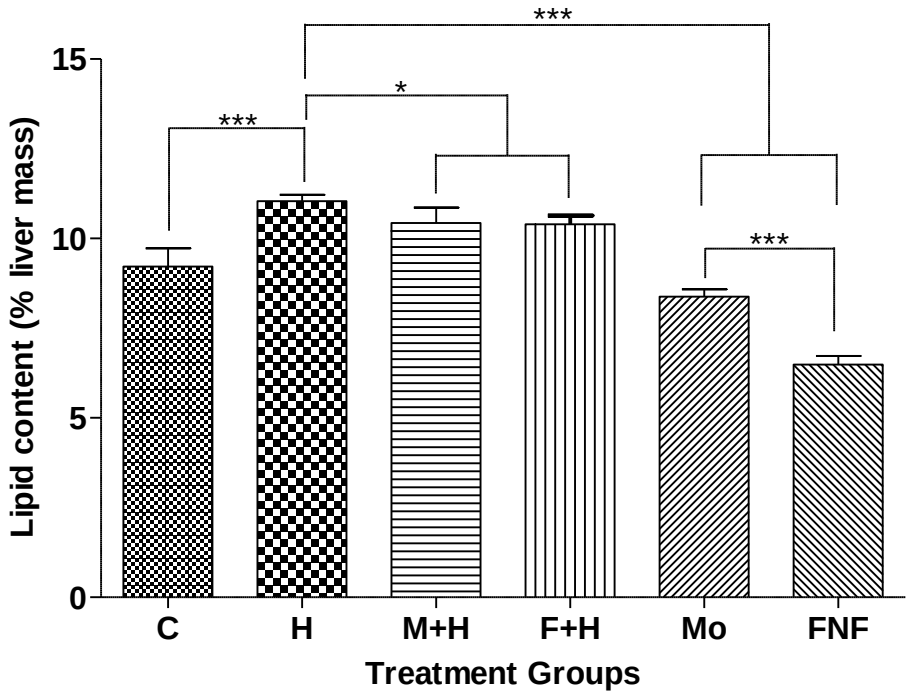

Figure 3.4A: Effects of methanolic extract of *Moringa oleifera* leaves on hepatic lipid storage of male Sprague Dawley rats.

Data was expressed as mean ± standard deviation. ***= significantly different at P<0.0001; *= significantly different at P<0.05; C= negative control (plain drinking water and plain gelatine cubes) (n= 19; 9 males, 10 females); H= 20% fructose solution and plain gelatine cubes (n= 18; 9 males, 9 females); M+H= 400mg.kg^{-1} of methanolic extract of *Moringa oleifera* leaves in gelatine cubes and 20% fructose solution (n= 17; 9 males, 8 females); F+H= 100 mg.kg^{-1} of fenofibrate in gelatine cubes and 20% fructose solution (n= 16; 8 males, 8 females); Mo= 400 mg.kg^{-1} of methanolic extract of *Moringa oleifera* leaves in gelatine cubes and plain drinking water (n= 16; 8 males, 8 females); FNF= 100 mg.kg^{-1} of fenofibrate in gelatine cubes and plain drinking water (n= 16; 8 males, 8 females).

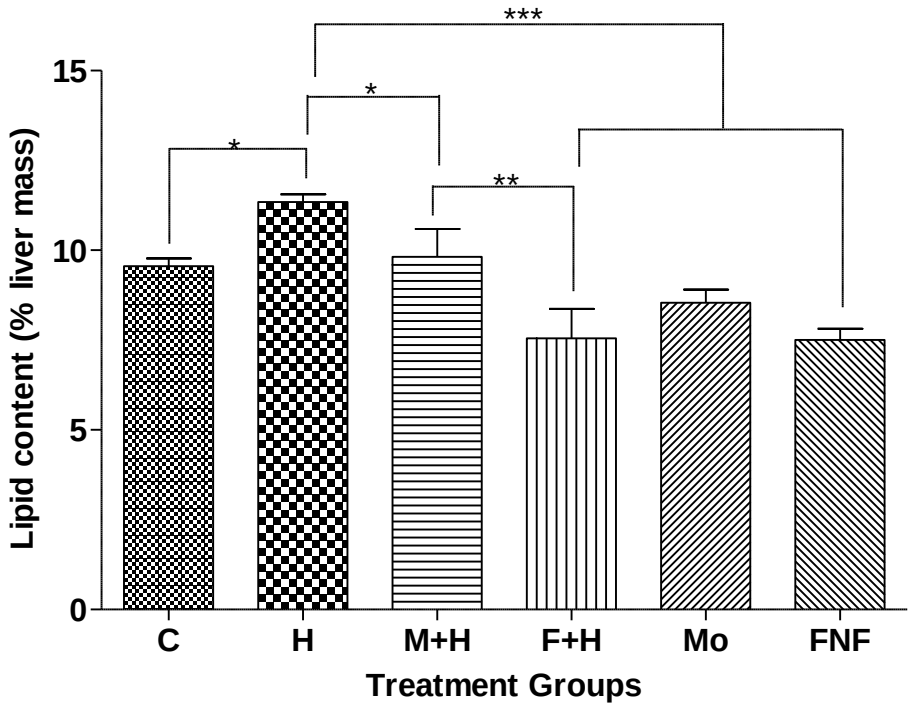

Figure 3.4B: Effects of methanolic extract of *Moringa oleifera* leaves on hepatic lipid storage of female Sprague Dawley rats.

Data was expressed as mean ± standard deviation. ***= significantly different at P<0.0001; **= significantly different at P<0.01; *= significantly different at P<0.05; C= negative control (plain drinking water and plain gelatine cubes) (n= 19; 9 males, 10 females); H= 20% fructose solution and plain gelatine cubes (n= 18; 9 males, 9 females); M+H= 400 $mg.kg^{-1}$ of methanolic extract of *Moringa oleifera* leaves in gelatine cubes and 20% fructose solution (n= 17; 9 males, 8 females); F+H= 100 $mg.kg^{-1}$ of fenofibrate in gelatine cubes and 20% fructose solution (n= 16; 8 males, 8 females); Mo= 400 $mg.kg^{-1}$ of methanolic extract of *Moringa oleifera* leaves in gelatine cubes and plain drinking water (n= 16; 8 males, 8 females); FNF= 100 $mg.kg^{-1}$ of fenofibrate in gelatine cubes and plain drinking water (n= 16; 8 males, 8 females).

3.4 Effects of methanolic leaf extract of *Moringa oleifera* on the lengths and masses of the gastro-intestinal tract (GIT) and accessory organs

3.4.1 The gastro-intestinal tract

Table 3.6 shows how the methanolic leaf extracts of *Moring aoleifera* affected the morphometry of the GIT organs of the study rats. In males, rats on combined fructose and fenofibrate had a significant decrease in absolute masses of the large intestine ($P<0.01$) and caecum ($P<0.05$) compared to control and *Moringa* alone groups. Those on combined fructose and *Moringa* had a significant decrease ($P<0.05$) in absolute mass of large intestine compared to control and *Moringa* alone groups. Rats receiving combined fructose and fenofibrate had a significant decrease ($P<0.05$) in relative masses of large intestine and caecum compared to those on *Moringa* alone. An insignificant difference was observed in both the absolute and relative masses of stomach and small intestine across the different treatment groups ($P>0.05$). No significant difference was observed in the lengths of small and large intestines across the different treatment groups ($P>0.05$).

In females, there were no significant differences in the absolute and relative masses of GIT visceral organs ($P>0.05$), as well as the lengths of small and large intestines ($P>0.05$) across all treatment groups.

In male rats the absolute and relative GIT organ masses and lengths were significantly greater than the females in corresponding groups ($P< 0.0001$).

Table 3.6: Effects of methanolic extract of *Moringa oleifera* leaves on the masses and lengths of GIT segments

Organ	Sex	C	H	M+H	F+H	Mo	FNF
Stomach (g)	Male	2.00±0.18^{a}	2.10±0.12^{a}	2.00±0.22^{a}	2.00±0.12^{a}	2.10±0.16^{a}	2.10±0.19^{a}
	Female	1.50±0.13$^{a\#}$	1.50±0.15$^{a\#}$	1.50±0.11$^{a\#}$	1.50±0.23$^{a\#}$	1.50±0.10$^{a\#}$	1.50±0.10$^{a\#}$
Stomach (grTL)	Male	0.05±0.00^{a}	0.05±0.00^{a}	0.05±0.01^{a}	0.05±0.00^{a}	0.05±0.00^{a}	0.05±0.01^{a}
	Female	0.04±0.00$^{a\#}$	0.04±0.01$^{a\#}$	0.04±0.00$^{a\#}$	0.04±0.01$^{a\#}$	0.04±0.00$^{a\#}$	0.04±0.00$^{a\#}$
S.I. (g)	Male	9.20±0.53^{a}	9.20±0.59^{a}	9.30±0.62^{a}	9.40±0.54^{a}	9.70±0.69^{a}	9.50±0.78^{a}
	Female	6.80±0.52$^{a\#}$	6.70±0.47$^{a\#}$	7.10±0.64$^{a\#}$	7.30±0.64$^{a\#}$	6.80±0.38$^{a\#}$	6.90±0.37$^{a\#}$
S.I. (grTL)	Male	0.23±0.01^{a}	0.23±0.01^{a}	0.23±0.02^{a}	0.24±0.02^{a}	0.24±0.02^{a}	0.24±0.02^{a}
	Female	0.18±0.02$^{a\#}$	0.19±0.01$^{a\#}$	0.19±0.02$^{a\#}$	0.20±0.02$^{a\#}$	0.19±0.01$^{a\#}$	0.19±0.01$^{a\#}$
S.I. (mm)	Male	1364±49.00^{a}	1367±64.00^{a}	1377±76.00^{a}	1360±67.00^{a}	1419±45.00^{a}	1373±57.00^{a}
	Female	1247±88.00$^{a\#}$	1229±103.00$^{a\#}$	1299±122.00$^{a\#}$	1233±44.00$^{a\#}$	1215±89.00$^{a\#}$	1259±73.00$^{a\#}$
L.I. (g)	Male	2.20±0.20^{a}	2.00±0.27^{ab}	1.90±0.10^{b}	1.80±0.13^{b}	2.30±0.30^{a}	2.00±0.15^{ab}
	Female	1.70±0.14$^{a\#}$	1.50±0.19$^{a\#}$	1.50±0.25$^{a\#}$	1.70±0.24$^{a\#}$	1.70±0.15$^{a\#}$	1.60±0.22$^{a\#}$
L.I. (grTL)	Male	0.05±0.01^{ab}	0.05±0.01^{ab}	0.05±0.00^{ab}	0.05±0.01^{a}	0.06±0.01^{b}	0.05±0.01^{ab}
	Female	0.05±0.01$^{a\#}$	0.04±0.01$^{a\#}$	0.04±0.01$^{a\#}$	0.05±0.01$^{a\#}$	0.05±0.00$^{a\#}$	0.05±0.01$^{a\#}$
L.I. (mm)	Male	249.00±8.80^{a}	236.00±18.00^{a}	238.00±13.00^{a}	248.00±10.00^{a}	254.00±14.00^{a}	244.00±9.20^{a}
	Female	221.00±19.00$^{a\#}$	213.00±6.70$^{a\#}$	214.00±18.00$^{a\#}$	219.00±8.30$^{a\#}$	225.00±9.30$^{a\#}$	221.00±21.00$^{a\#}$
Caecum (g)	Male	1.60±0.21^{a}	1.40±0.21^{ab}	1.40±0.39^{ab}	1.20±0.24^{b}	1.60±0.27^{a}	1.30±0.18^{ab}
	Female	1.10±0.19$^{a\#}$	1.00±0.16$^{a\#}$	0.97±0.12$^{a\#}$	1.10±0.23$^{a\#}$	1.10±0.16$^{a\#}$	1.00±0.14$^{a\#}$

Caecum (grTL)	Male	0.04±0.01^{ab}	0.03±0.01^{ab}	0.04±0.01^{ab}	0.03±0.01^{a}	0.04±0.01^{b}	0.03±0.01^{ab}
	Female	0.03±0.01$^{a\#}$	0.03±0.01$^{a\#}$	0.03±0.00$^{a\#}$	0.03±0.01$^{a\#}$	0.03±0.01$^{a\#}$	0.03±0.01$^{a\#}$

Data was expressed as mean ± standard deviation. ^{ab}= within row means with different superscript significantly different at P≤0.05; $^{\#}$= female rats had significantly lighter absolute and relative GIT organ masses, and shorter lengths than male rats (P< 0.0001); C= negative control (plain drinking water and plain gelatine cubes) (n= 19; 9 males, 10 females); H= 20% fructose solution and plain gelatine cubes (n= 18; 9 males, 9 females); M+H= 400 mg.kg^{-1} of methanolic extract of *Moringa oleifera* leaves in gelatine cubes and 20% fructose solution (n= 17; 9 males, 8 females); F+H= 100 mg.kg^{-1} of fenofibrate in gelatine cubes and 20% fructose solution (n= 16; 8 males, 8 females); Mo= 400 mg.kg^{-1} of methanolic extract of *Moringa oleifera* leaves in gelatine cubes and plain drinking water (n= 16; 8 males, 8 females); FNF= 100 mg.kg^{-1} of fenofibrate in gelatine cubes and plain drinking water (n= 16; 8 males, 8 females); grTL= mass relative to tibial length; S.I.= small intestines; L.I.= large intestines.

3.4.2 Accessory organs of digestion, kidneys and heart

Table 3.7 shows the sequelae of methanolic leaf extracts of *Moringa oleifera* on the accessory organs, heart and kidneys of the study rats. In males, rats receiving fenofibrate with or without fructose, had significantly higher ($P<0.0001$) absolute and relative masses of liver in comparison to other groups. The rats on fenofibrate alone had significantly heavier absolute kidney masses in comparison to those on fructose alone, combined fructose and *Moringa* ($P<0.01$) and negative control groups ($P<0.05$). Those receiving fructose alone had significantly lower ($P<0.01$) kidney masses relative to tibial length compared to those on combined fructose and fenofibrate. Rats on combined fructose and *Moringa* had significantly lower ($P<0.05$) kidney masses relative to tibial length compared to those on combined fructose and fenofibrate. The differences observed in the absolute and relative masses of heart and pancreas across different treatments were insignificant statistically ($P>0.05$).

In females, rats receiving fenofibrate with or without fructose, had absolute and relative liver masses that were significantly heavier ($P<0.0001$) in comparison to other groups. Rats on combined fructose and fenofibrate had significantly higher ($P<0.0001$) absolute kidney masses compared to control and those on fructose alone. Those on combined fructose and fenofibrate had significantly higher ($P<0.05$) absolute kidney masses compared to those on *Moringa* alone. Rats on combined fructose and fenofibrate had significantly higher ($P<0.05$) kidney masses relative to tibial length versus the control group. Insignificant differences were observed in the absolute and relative masses of heart and pancreas across different treatments ($P>0.05$).

In comparison for the measured organs, male rats absolute and relative accessory organ masses which were significantly greater ($P<0.0001$) in comparison to female rats.

Table 3.7: Effects of methanolic extract of *Moringa oleifera* leaves on the accessory organs of digestion, kidneys and heart

Organ	Sex	C	H	M+H	F+H	Mo	FNF
Heart (g)	Male	1.50±0.15^{a}	1.50±0.13^{a}	1.50±0.12^{a}	1.50±0.17^{a}	1.60±0.14^{a}	1.50±0.14^{a}
	Female	1.00±0.10$^{a\#}$	1.00±0.05$^{a\#}$	1.00±0.11$^{a\#}$	1.10±0.13$^{a\#}$	1.10±0.12$^{a\#}$	0.99±0.04$^{a\#}$
Heart (grTL)	Male	0.04±0.01^{a}	0.04±0.01^{a}	0.04±0.00^{a}	0.04±0.00^{a}	0.04±0.01^{a}	0.04±0.00^{a}
	Female	0.03±0.00$^{a\#}$	0.03±0.00$^{a\#}$	0.03±0.00$^{a\#}$	0.03±0.00$^{a\#}$	0.03±0.00$^{a\#}$	0.03±0.00$^{a\#}$
Liver (g)	Male	13.00±1.50^{a}	13.00±1.40^{a}	13.00±1.10^{a}	21.00±1.50^{b}	14.00±0.65^{a}	22.00±2.00^{b}
	Female	7.60±0.57$^{a\#}$	7.80±0.71$^{a\#}$	8.30±0.58$^{a\#}$	13.00±1.20$^{b\#}$	7.50±0.67$^{a\#}$	11.00±1.30$^{b\#}$
Liver (grTL)	Male	0.31±0.04^{a}	0.33±0.03^{a}	0.33±0.03^{a}	0.53±0.05^{b}	0.34±0.02^{a}	0.54±0.05^{b}
	Female	0.21±0.01$^{a\#}$	0.22±0.02$^{a\#}$	0.23±0.01$^{a\#}$	0.36±0.03$^{b\#}$	0.21±0.02$^{a\#}$	0.30±0.04$^{b\#}$
Pancreas (g)	Male	1.60±0.36^{a}	1.40±0.29^{a}	1.60±0.17^{a}	1.60±0.36^{a}	1.80±0.40^{a}	1.50±0.31^{a}
	Female	1.20±0.23$^{a\#}$	1.10±0.23$^{a\#}$	1.10±0.12$^{a\#}$	1.20±0.16$^{a\#}$	1.10±0.20$^{a\#}$	1.20±0.14$^{a\#}$
Pancreas (grTL)	Male	0.04±0.01^{a}	0.04±0.01^{a}	0.04±0.01^{a}	0.04±0.01^{a}	0.04±0.01^{a}	0.04±0.01^{a}
	Female	0.03±0.01$^{a\#}$	0.03±0.01$^{a\#}$	0.03±0.01$^{a\#}$	0.03±0.00$^{a\#}$	0.03±0.01$^{a\#}$	0.03±0.01$^{a\#}$
Kidneys (g)	Male	2.70±0.29^{a}	2.60±0.25^{a}	2.60±0.18^{a}	2.90±0.25^{ab}	2.90±0.29^{ab}	3.10±0.28^{b}
	Female	1.70±0.14$^{a\#}$	1.70±0.12$^{a\#}$	1.80±0.11$^{ab\#}$	2.00±0.11$^{b\#}$	1.70±0.11$^{a\#}$	1.80±0.10$^{ab\#}$
Kidneys (grTL)	Male	0.07±0.01^{ab}	0.06±0.01^{a}	0.07±0.01^{a}	0.08±0.01^{b}	0.07±0.01^{ab}	0.08±0.01^{ab}
	Female	0.05±0.0$^{a\#}$	0.05±0.00$^{ab\#}$	0.05±0.00$^{ab\#}$	0.05±0.00$^{b\#}$	0.05±0.01$^{ab\#}$	0.05±0.00$^{ab\#}$

Data was expressed as mean ± standard deviation. ^{ab}= within row means with different superscript significantly different at P≤0.05; $^{\#}$= female rats had significantly lower absolute and relative accessory organ masses compared to male rats (P<0.0001); C= control (plain drinking water and plain gelatine cubes) (n= 19; 9 males, 10 females); H= 20% fructose solution and plain gelatine cubes (n= 18; 9 males, 9 females); M+H= 400 $mg.kg^{-1}$ of methanolic extract of *Moringa oleifera* leaves in gelatine cubes and 20% fructose solution (n= 17; 9 males, 8 females); F+H= 100 $mg.kg^{-1}$ of fenofibrate in gelatine cubes and 20% fructose solution (n= 16; 8 males, 8 females); Mo= 400 $mg.kg^{-1}$ of methanolic extract of *Moringa oleifera* leaves in gelatine cubes and plain drinking water (n= 16; 8 males, 8 females); FNF= 100 $mg.kg^{-1}$ of fenofibrate in gelatine cubes and plain drinking water (n= 16; 8 males, 8 females); grTL= mass relative to tibial length.

3.5 Impact of methanolic leaf extract of *Moringa oleifera* on biochemical markers of general health

3.5.1 Liver enzymes

Table 3.8 shows the impact of methanolic leaf extracts of *Moringa oleifera* on liver enzymes; alanine amino transferase (ALT) and alkaline phosphatase (ALKP). In males, rats receiving fenofibrate with or without fructose, had significantly greater ($P<0.0001$) ALKP activity in comparison to other groups. However, there was an insignificant difference ($P>0.05$) in the activity of ALT amongst the different groups.

In females, rats on combined fructose and fenofibrate had significantly higher ALKP activity compared to negative control ($P<0.01$), those on *Moringa* alone ($P<0.01$) those on combined fructose and *Moringa* ($P<0.01$), and those on fructose alone ($P<0.05$). Those on fenofibrate alone had significantly greater ($P<0.05$) ALKP activity compared to either those on *Moringa* alone or those on combined fructose and *Moringa*. The female rats on combined fructose and fenofibrate also had significantly higher ($P<0.05$) ALT activity compared to those on *Moringa* alone.

Comparatively, the male rats had significantly higher ($P<0.0001$) ALT and ALKP activity compared to female rats.

Table 3.8: Effects of methanolic extract of *Moringa oleifera* leaves on liver enzymes

Parameter	Sex	C	H	M+H	F+H	Mo	FNF
ALT ($U.l^{-1}$)	Male	105.00 ± 34.00^{a}	82.00 ± 67.00^{a}	57.00 ± 8.90^{a}	76.00 ± 5.70^{a}	79.00 ± 36.00^{a}	92.00 ± 30.00^{a}
	Female	$58.00\pm3.20^{ab\#}$	$53.00\pm4.90^{ab\#}$	$53.00\pm1.90^{ab\#}$	$60.00\pm6.80^{a\#}$	$51.00\pm3.10^{b\#}$	$58.00\pm9.30^{ab\#}$
ALKP ($U.l^{-1}$)	Male	129.00 ± 22.00^{a}	148.00 ± 32.00^{a}	159.00 ± 43.00^{a}	295.00 ± 67.00^{b}	155.00 ± 53.00^{a}	285.00 ± 61.00^{b}
	Female	$107.00\pm33.00^{ac\#}$	$109.00\pm30.00^{ac\#}$	$96.00\pm15.00^{a\#}$	$174.00\pm55.00^{b\#}$	$98.00\pm12.00^{a\#}$	$159.00\pm49.00^{bc\#}$

Data was expressed as mean ± standard deviation. ^{abc}= within row means with different superscripts significantly different at P≤0.05; $^{\#}$= female rats had significantly lower ALT and AKLP concentrations compared to male rats (P<0.0001); C= negative control (plain drinking water and plain gelatine cubes) (n= 19; 9 males, 10 females); H= 20% fructose solution and plain gelatine cubes (n= 18; 9 males, 9 females); M+H= 400 $mg.kg^{-1}$ of methanolic extract of *Moringa oleifera* leaves in gelatine cubes and 20% fructose solution (n= 17; 9 males, 8 females); F+H= 100 $mg.kg^{-1}$ of fenofibrate in gelatine cubes and 20% fructose solution (n= 16; 8 males, 8 females); Mo= 400 $mg.kg^{-1}$ of methanolic extract of *Moringa oleifera* leaves in gelatine cubes and plain drinking water (n= 16; 8 males, 8 females); FNF= 100 $mg.kg^{-1}$ of fenofibrate in gelatine cubes and plain drinking water (n= 16; 8 males, 8 females); ALT= alanine aminotransferase; ALKP= alkaline phosphatase.

3.5.2 Markers of kidney function

Table 3.9 shows the effect of methanolic leaf extract of *Moringa oleifera* on biomarkers of kidney function (creatinine, blood urea nitrogen (BUN) and urea to creatinine ratio). In males, rats receiving fenofibrate with or without fructose, had significantly higher ($P<0.0001$) plasma BUN concentrations and urea to creatinine ratio compared to those on fructose alone or combined fructose and *Moringa*. However, those on fenofibrate alone had significantly less ($P<0.05$) concentration of creatinine in plasma compared with those on combined fructose and *Moringa*.

In females, compared to the negative controls a significant decrease in BUN concentrations compared to those on *Moringa* alone ($P<0.0001$), fructose alone ($P<0.01$) and combined fructose and FNF ($P<0.05$) was recorded. A significantly lower ($P<0.01$) urea to creatinine ratio of the rats on either fructose alone ($P<0.01$) or *Moringa* alone ($P<0.05$) in comparison to the negative controls was seen. The differences ($P>0.05$) in the plasma creatinine concentrations of female rats across different treatment groups were insignificant ($P>0.05$).

Male rats had significantly higher ($P<0.0001$) plasma BUN concentrations and urea to creatinine ratio than female rats, but no sex difference in plasma creatinine concentrations ($P>0.05$) was noted.

Table 3.9: Effects of methanolic extract of *Moringa oleifera* leaves on markers of kidney function

Parameter	Sex	C	H	M+H	F+H	Mo	FNF
Creatinine ($mg.dl^{-1}$)	Male	0.59 ± 0.12^{ab}	0.61 ± 0.11^{ab}	0.65 ± 0.05^{a}	0.58 ± 0.07^{ab}	0.55 ± 0.05^{ab}	0.51 ± 0.06^{b}
	Female	0.58 ± 0.05^{a}	0.61 ± 0.06^{a}	0.58 ± 0.07^{a}	0.60 ± 0.11^{a}	0.60 ± 0.11^{a}	0.54 ± 0.09^{a}
BUN ($mg.dl^{-1}$)	Male	20.00 ± 3.30^{ab}	17.00 ± 3.90^{a}	17.00 ± 2.90^{a}	24.00 ± 2.70^{b}	20.00 ± 2.30^{ab}	24.00 ± 2.10^{b}
	Female	$19.00\pm1.60^{a\#}$	$16.00\pm2.30^{b\#}$	$17.00\pm2.10^{ab\#}$	$16.00\pm2.00^{b\#}$	$15.00\pm1.20^{b\#}$	$17.00\pm2.20^{ab\#}$
Ur:Cr^{-1}	Male	36.00 ± 13.00^{ab}	29.00 ± 9.40^{ac}	26.00 ± 4.30^{a}	42.00 ± 7.70^{bc}	37.00 ± 7.30^{ab}	48.00 ± 7.60^{b}
	Female	$34.00\pm5.40^{a\#}$	$25.00\pm2.80^{b\#}$	$29.00\pm3.70^{ab\#}$	$27.00\pm5.20^{ab\#}$	$26.00\pm5.10^{b\#}$	$32.00\pm4.60^{ab\#}$

Data was expressed as mean ± standard deviation. ^{abc}= within row means with different superscript significantly different at P≤0.05; $^{\#}$= female rats had significantly lower plasma BUN concentrations and urea to creatinine ratio than male rats (P<0.0001); C= negative control (plain drinking water and plain gelatine cubes) (n= 19; 9 males, 10 females); H= 20% fructose solution and plain gelatine cubes (n= 18; 9 males, 9 females); M+H= 400 $mg.kg^{-1}$ of methanolic extract of *Moringa oleifera* leaves in gelatine cubes and 20% fructose solution (n= 17; 9 males, 8 females); F+H= 100 $mg.kg^{-1}$ of fenofibrate in gelatine cubes and 20% fructose solution (n= 16; 8 males, 8 females); Mo= 400 $mg.kg^{-1}$ of methanolic extract of *Moringa oleifera* leaves in gelatine cubes and plain drinking water (n= 16; 8 males, 8 females); FNF= 100 $mg.kg^{-1}$ of fenofibrate in gelatine cubes and plain drinking water (n= 16; 8 males, 8 females); BUN= blood urea nitrogen; Ur:Cr^{-1}= urea to creatinine ratio.

CHAPTER FOUR- DISCUSSION

The consequences of methanolic leaf extracts of *Moringa oleifera* on metabolic derangement secondary to excess fructose consumption were investigated in this study. The objectives specifically targeted the effects on growth performance, the development of metabolic dysfunction, the gastro-intestinal tract and accessory organs and general health profile of the study rats of both sexes.

Administration of 20% fructose solution significantly elevated hepatic lipid content in both sexes, and triglycerides concentrations in female rats compared to negative controls. MEMO leaves (400 $mg.kg^{-1}$ body weight) prevented the effect of fructose on hepatic and circulating lipids. Fenofibrate treated groups from both sexes had hepatomegaly, higher fasting blood glucose and higher alkaline phosphatase compared to negative controls. No adverse effects were observed with fructose, *Moringa* or fenofibrate on growth performance and other general health markers. Generally, male rats had significantly higher levels of most of the metabolic parameters than their corresponding females.

4.1 Growth performance

High-fructose diet consumption overtime causes excess body mass gain (Tappy *et al.*, 2010) and abdominal visceral fat deposition (Crescenzo *et al.*, 2014) both of which increase the risk of metabolic dysfunction. Body mass gain and visceral fat deposition can be assessed by measuring the BMI and WC (Ahmed *et al.*, 2014; Crescenzo *et al.*, 2014). In this study, fructose consumption did not induce any significant effect on body mass gain, BMI and WC in both sexes (Figures 3.1A, 3.1B, 3.2A, 3.2B and Table 3.1). The insignificant effect of fructose on body mass gain, BMI and WC was in contrast to what Mamikutty *et al.* (2014) observed following administration of similar 20% fructose solution on adult Wistar rats for eight weeks. The differences could be attributed to breed and age differences of the study rats (Ghezzi *et al.*, 2012).

Nevertheless, all the rats had gained significant body mass following the ten weeks treatment period, with males having significantly higher body masses compared to their female counterparts (Figures 3.1A, 3.1B, 3.2A, 3.2B and Table 3.1). This could be due to high testosterone levels in males that enhance the activity of growth hormone, and promote the synthesis of proteins (Gibney *et al.*, 2003).

Fenofibrate significantly lowered the body masses of male rats irrespective of whether they had fructose or not. This agrees with Ji *et al.* (2005) who also made similar observations in male Sprague Dawley rats. This could be due to the lipid lowering ability of fenofibrate (Mancini *et al.*, 2001). However, no significant effect was induced by fenofibrate on body mass gain, BMI and WC of female rats across all treatment groups in this study. Sex specific responses to fibrates between male and female SD rats have been reported (Jalouli *et al.*, 2003). Similarly the effect of fenofibrate on body mass was more pronounced in males than females (Yoon *et al.*, 2002). It is not unusual to find sex-specific responses to drugs (Franconi *et al.*, 2007).

Male rats had higher mass gains, BMI and WC compared to their corresponding females. This could also be attributed to the effect of testosterone at puberty (Gibney *et al.*, 2003) as stated earlier.

Body mass is not a good indicator of growth performance as it can be affected by food substances in the GIT and fluid in the bladder, thus affecting the overall body mass of the animal (Stookey, 2016). Although the rats were fasted they still had residual content in the GITs. Empty carcass mass thus provides a better indicator of growth performance than intact body mass since the effect of GIT filling is avoided (Owens *et al.*, 1995). Administration of 20% fructose solution did not affect the empty carcass masses of the male rats. Male rats on combined fructose and *Moringa* had significantly lower empty carcass masses compared to those on *Moringa* alone, but there was no significant difference in the empty carcass masses of negative control, fructose and *Moringa* treated groups. It could thus be speculated that *Moringa* had not exerted any effect in the empty carcass masses of the male rats. However, fenofibrate had a negative effect on the empty carcass masses of the male rats (Table 3.1). So this reinforced the findings in the intact body mass stated earlier, and negated the potential impact of gut fill. No significant effect was exerted by fructose, *Moringa* or fenofibrate on the empty carcass masses of female rats.

Male rats had heavier empty carcass masses compared to their corresponding females, which could be due to the effect of testosterone at puberty (Gibney *et al.*, 2003).

Linear growth, especially the tibial length, is a better indicator of long term growth performance than intact and empty carcass mass. The length of the bones during the growing phase is not subject to hyperacute changes which can occur to body mass. There is also a dose dependent effect of growth hormone on long bones (Eshet *et al.*, 2004). It has been reported that long bones (femur and tibia) morphometry is not adversely affected by fructose administration even though some studies suggested that sugar intake has a negative effect on bone mineralization in growing animals (Tsanzi *et al.*, 2008). In this study, the lengths, masses and Seedor indexes of the femora and tibiae were not significantly affected by fructose across different treatments in both sexes (Table 3.2). This is similar to what was reported by Tsanzi *et al.* (2007) and Tsanzi *et al.* (2008) following administration of fructose-sweetened beverages to female SD rats. *Moringa* administration exerted no effect on the lengths, masses and Seedor indexes of the long bones in male rats compared to negative control and fructose treated groups in this study. Although fenofibrate was reported to have a preventive effect on bone morphometry by studies that used osteoblast cultures of SD rats (Patel *et al.*, 2014; Stunes *et al.*, 2011), a negative effect of fenofibrate was recorded on linear growth in this study. This discrepancy might be due to culture system used in those studies instead of whole organism. Seedor index (which has no unit) indicates the density of a bone; the greater the Seedor index the denser the bone (Almeida *et al.*, 2008). Although the tibial Seedor index shows a difference in the males (F+H vs Mo) (Table 3.2), a subjective visual assessment of the radiographs did not show such difference (Figure 3.3A).

Except for the negative control that had higher tibial length than fructose treated groups, insignificant differences were noted in the linear growth of female rats across the different treatments.

The lengths, masses and Seedor indexes of femora and tibiae of the males were greater compared to females in matched groups. This could probably be attributed to the increased activity of growth hormone secondary to high testosterone levels at puberty (Gibney *et al.*, 2003).

4.2 Development of metabolic dysfunction

4.2.1 Glucose, triglycerides and cholesterol concentrations in circulation

Both short and long term consumption of excess dietary fructose in rodents can lead to elevated plasma triglycerides concentrations and hepatic lipid synthesis (Lê and Tappy, 2006). Plasma glucose homeostasis is also affected by excess fructose consumption (Lê and Tappy, 2006). However, in the current study we observed that no significant effect was exerted by either fructose or *Moringa* on fasting blood glucose (FBG) in both sexes (Table 3.3). Although a previous study reported a reduction in FBG following administration of 400 $mg.kg^{-1}$ of MEMO leaves to Wistar rats of both sexes for 49 days (Bais *et al.*, 2014), Zvinorova *et al.* (2015) observed no effect on FBG following administration of dietary *Moringa oleifera* leaf powder (MOLP) to SD rats for five weeks. This could be attributed to the type of diet and/or specie of rats used in the study. Although male rats had higher FBG than females, the FBG was higher in rats that had fenofibrate than other groups in both sexes. This might be due to the effect of fenofibrate which causes inflammation and oxidative stress to the pancreas, impairing insulin secretion and glucose homeostasis (Liu *et al.*, 2011).

The effect of fructose, *Moringa* and fenofibrate on triglycerides (TGs) and cholesterol was not significant in male rats. However, in females, higher TGs concentrations were recorded in fructose treated groups compared to negative controls (Table 3.3). This was prevented following administration of MEMO leaves (400 $mg.kg^{-1}$ body weight). It is notable that the effects of fructose and *Moringa* are in agreement with a previous study that observed similar effects when an aqueous leaf extract of *Moringa oleifera* was given (200 $mg.kg^{-1}$ body weight) to adult Wistar rats fed 66% fructose diet for 60 days (Divi *et al.*, 2012). The observed effect of *Moringa* on TGs could be due to its phytochemicals that inhibit fat utilization and HMG-Co-A reductase, decrease LDL-c and increase HDL-c concentrations (Ahmed *et al.*, 2014; Divi *et al.*, 2012;Ghasi *et al.*, 2000). However, when we undertook the current study we did not measure activity of HMG-Co-A reductase and the subtypes of cholesterol. Male rats had higher FBG, but lower triglycerides and cholesterol concentrations compared to female rats. This could be due to the fact that female rats are more prone to fructose-induced lipid abnormalities than male rats (Vilà *et al.*, 2010). The higher concentration of cholesterol in female rats has been attributed to

the effect of oestrogen which increases circulating high density lipoprotein cholesterol (Kumar *et al.*, 2010).

4.2.2 Insulin and HOMA-IR index

The metabolism of carbohydrates and lipids is regulated by insulin, a hormone that maintains normoglycaemia by enhancing glucose uptake in peripheral tissues (Wilcox, 2005). Homeostasis Model of Assessment of Insulin Resistance (HOMA-IR) index is used to assess insulin sensitivity; the higher the index the lower the insulin sensitivity (Divi *et al.*, 2012). High dietary fructose intake is linked with elevations in plasma C-peptide levels that affects insulin sensitivity thereby leading to insulin resistance (Lê and Tappy, 2006). The dietary fructose also interferes with insulin receptor tyrosine phosphorylation in the brain leading to insulin resistance (Lê and Tappy, 2006). In the current study, insignificant differences in the insulin concentrations and the HOMA-IR indexes were observed across all treatment groups (Table 3.4). This showed that drinking a 20% fructose solution did not result in insulin-mediated metabolic dysfunction. This could be due to the method of fructose administration and the age of the rats since high-fructose diets were shown to cause metabolic alterations more than fructose in drinking water (Lê and Tappy, 2006). The male rats had higher concentrations of insulin in serum and HOMA-IR indexes compared to corresponding females. It has been shown that Insulin resistance is higher in adult males than females, and is thought to be due to the effect of insulin and growth hormone during growth period (Kurtoglu *et al.*, 2010).

4.2.3 Visceral fat and epididymal fat pad masses

It has been shown that fructose-rich diets enhance visceral fat and epididymal fat deposition, and this predisposes to the developing insulin resistance and metabolic syndrome (Crescenzo *et al.*, 2014). The current study observed that no significant effect was induced by either fructose or *Moringa* in both sexes (Table 3.5). In males, rats receiving combined fructose and fenofibrate had a significantly lower absolute visceral fat and that relative to tibial length compared to those that had fructose alone or *Moringa* alone. While the differences in absolute epididymal fat masses were insignificant among all the groups, in males, fenofibrate had a negative effect on the relative masses of the epididymal fat of the male rats. This is due to the fact that fenofibrate enhances the

expression of PPARα genes responsible for oxidation of fatty acids in epididymal fatty tissues (Jeong and Yoon, 2009).

In female rats, both the absolute and relative visceral fat masses were low only in rats receiving fenofibrate alone compared to those on combined fructose and *Moringa*; otherwise no significant difference was observed in other groups.

In comparison, female rats had significantly higher absolute visceral fat and that relative to tibial length than their male counterparts. This could be due to the effect of oestrogen which increases adiposity in females, and is found in higher concentrations in post pubertal females than males (Power and Schulkin, 2008; Williams, 2004).

4.2.4 Hepatic lipid storage

The liver is the major site of fructose metabolism thereby making it vulnerable to storage of phospho-lipids, triacylglycerols and fatty acids (Bray *et al.*, 2004). It has been shown that high-fructose diets stimulate intrahepatic lipid accumulation (Tappy *et al.*, 2010; Stanhope *et al.*, 2009) leading to non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) (De Castro *et al.*, 2013). NAFLD is characterized by hepatic accumulation of fat in non-alcoholics without history of liver disease, and its progressive form is known as NASH (Dabhi *et al.*, 2008; Georgescu and Georgescu, 2007). NAFLD is thought to be the liver manifestation of metabolic syndrome due to its association with diabetes mellitus, increased adiposity, hyperlipidaemia and insulin resistance (Dabhi *et al.*, 2008; Georgescu and Georgescu, 2007). The worldwide increasing prevalence is about 74% amongst obese diabetics (Dabhi *et al.*, 2008). NAFLD is a major originator of chronic hepatic pathology and hepatocellular carcinoma (Georgescu and Georgescu, 2007). It starts by abnormally depositing fat in the liver, progresses to NASH due to accompanying inflammatory changes and consequently develops fibrotic and cirrhotic changes (Dabhi *et al.*, 2008).

The result of current study showed that 20% fructose solution had induced a significant increase in hepatic lipids in comparison to control groups in the two sexes of the rats (Figures 3.4A and 3.4B). This is not surprising since hepatic lipid content increases within 6 weeks of high-fructose diet consumption in rats (Tappy *et al.*, 2010). However, a major and important finding for this study was that administration of MEMO leaves (400

mg.kg^{-1} body weight) was able to prevent the effect of fructose on hepatic lipid stores. The prophylactic effect of *Moringa oleifera* might be attributed to β-sitosterol which indirectly decreases cholesterol levels (Divi *et al.*, 2012; Ghasi *et al.*, 2000), as well as saponins and tannins that inhibit dietary fat utilization (Ahmed *et al.*, 2014).

The effect of fenofibrate was more pronounced than that of *Moringa*. Fenofibrate was able to decrease hepatic lipid storage possibly due its lipid lowering effect via binding to its nuclear receptor and inhibiting lipid metabolism (Ji *et al.*, 2005). This has also been shown by Ferreira *et al.* (2008) where administration of fenofibrate (100 mg.kg^{-1} body weight) had reduced hepatic lipid synthesis and storage in Wistar rats.

Although it was previously shown that female SD rats were more prone to fructose-induced metabolic derangements including hepatic lipid abnormalities than males (Vilà *et al.*, 2010), current study observed no significant difference in hepatic lipid content between male and female rats. This could be due to depletion of hepatic lipid stores secondary to eight hours overnight fast before terminating the rats.

4.3 Lengths and masses of the gastro-intestinal tract and vital organs

4.3.1 The gastro-intestinal tract

The growth of an animal depends on the digestive and absorptive capacity of the GIT to deliver nutrients to the tissues (Zvinorova *et al.*, 2015). The growth performance of an animal can be affected by phytotherapy via altering the GIT morphometry (Pérez *et al.*, 2007). Our study showed that administration of 20% fructose solution alone had no effects on GIT visceral organs in both sexes (Table 3.6). In males, absolute masses of the large intestines and caeca were significantly lower in rats that had combined fructose and *Moringa* or fenofibrate than negative control and *Moringa* alone treated groups; but the masses relative to tibial length were significantly lower in those that had combined fructose and fenofibrate than those on *Moringa* alone. This could be attributed to the lipid lowering effect of fenofibrate on the GIT which could have been achieved by decreasing lipid absorption across the apical membrane of the enterocytes (Valasek *et al.*, 2007). Since there was no significant difference between the absolute and relative GIT visceral masses of negative controls and *Moringa* alone treated groups,

it could be speculated that no significant effect was induced by administration of MEMO leaves on the GIT visceral organs.

The caecum plays a major role in rats through fermentation of polysaccharides to short chain fatty acids, and this generates more energy for body requirements (Zvinorova *et al.*, 2015; Pascoal *et al.*, 2013). The heavier caecal mass observed in *Moringa* treated groups in this study could be due to the polysaccharides present in the methanolic extract of the plant, which could have increased the number of caecal mucosal cells following fermentation process (Dangarembizi *et al.*, 2014). The heavy caecal mass could have also developed during the early development of the rats' caecum since the study was commenced immediately post-weaning (Zvinorova *et al.*, 2015). Similar effects on the caecum were also observed with plant extracts by other investigators (Zvinorova *et al.*, 2015; Beya *et al.*, 2012; Erlwanger and Cooper, 2008).

The significantly greater absolute and relative GIT organ weights, and span of the male rats compared to their matched females was probably due to the effects of testosterone. Testosterone concentrations are normally higher in mature males compared to females and testosterone promotes tissue growth (Gibney *et al.*, 2003).

4.3.2 Vital and accessory organs

High fructose diets have been shown to cause cardiovascular, renal and hepatic complications that affect their individual organ masses (Dachani *et al.*, 2012). Although there is a belief that 'natural is better than synthetic', phytotherapy is not free from unwanted side effects (Oyagbemi *et al.*, 2013). Hepatic and kidney damage was reported following chronic administration of MEMO leaves (400 $mg.kg^{-1}$ body weight) to adult Wistar rats for eight weeks (Oyagbemi *et al.*, 2013). Fenofibrate treatment is associated with hepatomegaly (Ji *et al.*, 2005). Hence it was important to assess the vital organs in this study. Administration of 20% fructose solution in this study had no impact on the absolute and relative masses of the heart, pancreas, liver and kidney in both sexes (Table 3.7). No significant effect was observed in the masses of these organs in *Moringa* treated groups in both sexes. However, the absolute and relative masses of the liver and kidneys were higher in the fenofibrate groups of both sexes. This is not surprising for the liver as fenofibrate treatment has been shown to cause hepatomegaly in male SD rats (Hong *et al.*, 2007; Ji *et al.*, 2005), a finding which has

been attributed to the inflammatory effect of fenofibrate on the liver (Tolman, 2000). The increase in kidney mass seen in fenofibrate treated groups could be attributed to adverse drug reaction of fenofibrate on the kidney, since the drug has been shown to be nephrotoxic even in subjects without renal insufficiency (Attridge *et al.*, 2013).

As expected, the male rats had significantly higher absolute and relative accessory organ masses compared to female rats, which is probably due the effects of testosterone at puberty as stated earlier.

4.4 General health profile

When undertaking interventional studies, it is important to monitor the health of the animals by assessing the toxicity, safety and the risks associated with the interventions (Oyagbemi *et al.*, 2013). In view of this, the surrogate markers of function of vital metabolic organs, specifically the liver and kidney were assessed in this study.

4.4.1 Liver enzymes

The enzyme alanine amino transferase (ALT), found in the liver cells , is a useful marker in conditions associated with liver damage (Sharifudin *et al.*, 2013; Rajesh *et al.*, 2009). The enzyme alkaline phosphatase (ALKP) is found in the cells lining the liver biliary ducts and it abnormally rises in conditions associated with bile duct damage/obstruction. Unlike ALT, ALKP is not specific to the liver as its levels are also elevated in conditions associated with osteoblast activity and pregnancy (Schoch and Whiteman, 2007). ALKP is also produced by intestinal cells of the GIT (Thapa and Walia, 2007).

A strong relationship exists between the components of metabolic syndrome, NAFLD and NASH (Kerner *et al.*, 2005). It has been reported that prolonged ingestion of high-fructose diets predisposes to NAFLD in rats (De Castro *et al.*, 2013). These liver diseases are associated with abnormal high levels of ALT and to a lesser extent, ALKP (Kerner *et al.*, 2005) which are the surrogate markers of liver function. In this study, administration of 20% fructose solution did not affect the plasma concentrations of either ALT or ALKP in both sexes (Table 3.8). However, it was observed that in both sexes, rats on fenofibrate with or without fructose had higher concentrations of ALKP compared to other groups. This was probably be due to the effect of fenofibrate which increases osteoblast activity leading to elevated plasma ALKP concentrations (Syversen *et al.*,

2009). However, hepatomegaly was also observed in fenofibrate treated groups in this study as stated earlier; thus it is unclear what the source was of the ALKP as the assay used was unable to differentiate between the isoforms of ALKP.

4.4.2 Markers of kidney function

Increased plasma concentrations of urea and creatinine as well as urea to creatinine ratio have been considered as indicators of renal damage (Oyagbemi *et al.*, 2013). Previous studies have shown that chronic fructose ingestion predisposes to renal dysfunction (Nakayama *et al.*, 2010). Administration of MEMO leaves (200 $mg.kg^{-1}$ and 400 $mg.kg^{-1}$) to adult male Wistar rats for eight weeks was reported to predispose to kidney damage as corroborated by higher than normal urea nitrogen in blood (BUN) as well as creatinine (Oyagbemi *et al.*, 2013). This is in variance with the data from this study which showed that neither administration of 20% fructose solution nor that of MEMO leaves (400 $mg.kg^{-1}$) for ten weeks caused any renal toxicity as assessed by creatinine and urea in Sprague Dawley rats of both sexes. This is evident by the relative decrease in BUN, creatinine and urea to creatinine ratio in both the fructose and *Moringa* treated groups compared to negative controls (Table 3.9). Thus whilst the *Moringa* extract used in the current study could be safe on the kidneys as there was no alteration in the markers of renal function, the fenofibrate treated groups had significantly elevated plasma BUN and urea to creatinine ratio in males. This could be due to the effects of fenofibrate on glomerular filtration rate (GFR) which has been known to alter serum BUN concentrations in human subjects (Chen *et al.*, 2011).

In this study, it was observed that male rats had significantly higher plasma BUN concentrations and urea to creatinine ratio than female rats. This might be due to lower muscle mass seen in females compared to male rats (Pagana, 2013).

CHAPTER FIVE- CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study investigated the effects of methanolic leaf extracts of *Moringa oleifera* on fructose-induced metabolic dysfunction in both sexes of growing Sprague Dawley rats.

We used unrestricted availability of 20%fructose solution for drinking which has been shown to be an effective experimental intervention in rodent models for the development of metabolic dysfunction (Mamikutty *et al.*, 2014). However, in the current study, metabolic syndrome did not fully develop and this could be due to utilization of dietary nutrients for growth during the rapid growth period of the rats.

Administration of 20% fructose solution to rats for 10 weeks elevated hepatic lipid content (both sexes) and triglycerides concentrations (female rats) without affecting growth performance, organ dimensions, adiposity and other surrogate biomarkers of health of the animals.

An exciting finding was that the elevation in triglycerides and hepatic lipid content were prevented by oral provision of methanolic extracts of *Moringa oleifera* leaves.

There were no adverse effects observed with the administration of methanolic leaf extracts of *Moringa oleifera.* On the contrary, fenofibrate treated groups (both sexes) developed hepatomegaly, higher fasting blood glucose and higher alkaline phosphatase levels. This shows that the use of phytomedicines like *Moringa oleifera* leaves extracts would be a better alternative in managing the components of metabolic syndrome than synthetic agents like fenofibrate.

Generally, male rats had higher metabolic parameters than their corresponding females except for triglycerides, cholesterol and visceral fat pad where the reverse case was observed. This shows that studies should not be restricted to just one sex.

In addition, there were some differences noted in the growing animals compared to adults.

5.2 Limitations and recommendations

Additional measures that could have been included to further elucidate the effects of fructose, *Moringa* and fenofibrate in this study, should be considered in future studies.

5.2.1 Further assessment of metabolic syndrome

Studies on metabolism usually include oral glucose tolerance test (OGTT). However whilst the OGTT would have shown the ability of the rats to handle an oral glucose load, it was not included as part of the study as the test is not as effective as HOMA-IR.

Concentrations of leptin, LDL, VLDL and HDL (which are also markers of metabolic syndrome) could not be determined, through which the dyslipidaemic features of the markers would have been observed.

Blood pressure, specifically the presence of hypertension which is a major component of metabolic syndrome, could not be measured.

Thus to assess features of metabolic syndrome further, mixed diets (like fructose solution and cholesterol or other fat diets) should be considered in future studies. This would increase the hepatic lipid deposition and subsequently full blown metabolic syndrome. High-fructose diet has been reported to cause metabolic dysfunction more than fructose drinking water, and thus should be considered. There is need for molecular studies on metabolic alterations due to intake of high fructose diets in growing rats.

The present study had used methanolic extract of *Moringa oleifera* leaves; future studies should compare the effects of different extracts of the plant leaves (such as methanolic and aqueous or methanolic and ethanolic) on fructose-induced metabolic dysfunction. This would show which extract is more effective since the phytochemical composition depends on the solvent used in extracting the plant (Kasolo *et al.*, 2010).

5.2.2 Further morphological measurements

Histology of the liver (NASH/NAFLD scoring), kidneys, pancreas and intestines was not done and it might have revealed ultrastructural damage. These tissues have a great functional reserve capacity, therefore ultrastructural damage may have been present but surrogate markers of function tend to only change from normal physiological levels when there is severe structural damage to these tissues (Oyagbemi *et al.*, 2013; Fakurazi *et al.*, 2008a; Pari and Kumar, 2002). Therefore for further safety evaluation, histology of these tissues needs to be considered in future studies especially the liver and kidneys

since enlargement of these organs was observed in fenofibrate treated groups in this study.

5.2.3 Assessment of bones

Radiographs were taken and attempts made to assess the bone density subjectively. The Seedor index determination does not give exact density of a bone but only indicates relative bone density (Almeida *et al.*, 2008), a more objective measure would be to use densitometry.

In addition, as an adjunct to the Seedor index, actual bone mineralization could have been determined from the ash content and assaying the amount of specific minerals including calcium.

Future studies should also consider the determination of ALKP isotypes as they were not determined in this study.

5.2.4 Phytochemical analysis of the plant extracts

Although the chemical constituents of *Moringa oleifera* plant extracts are the determinants of its medicinal properties (Sánchez-Machado *et al.*, 2010), but they were not analyzed in this study. Hence future studies should assay the phytochemicals in the plant extracts.

CHAPTER SIX- REFERENCES

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APPENDICES

APPENDIX 1: Ethics clearance certificate

STRICTLY CONFIDENTIAL

ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2015/11/51/B

APPLICANT: Mr N Muhammad

SCHOOL: Physiology
DEPARTMENT:
LOCATION:

PROJECT TITLE: Effects of methanolic extract of Moringa oleifera leaves on fructose-induced metabolic dysfunction in growing Wistar Rats

Number and Species

60 Male Wistar Rats and 60 Female Wistar Rats

Approval was given for the use of animals for the project described above at an AESC meeting held on 2015/11/24. This approval remains valid until 2017/12/01.

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

None

Signed: _____ (Chairperson, AESC) Date: 1st Dec 2015

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: 1 December 2015

cc: Supervisor: Professor K Erlwanger
Director: CAS

Works 2000/Iain0015/AESCCert.wps

APPENDIX 2: Ethics clearance modification

AESC 2012 M&E

Please note that only typewritten applications will be accepted.

UNIVERSITY OF THE WITWATERSRAND

ANIMAL ETHICS SCREENING COMMITTEE
MODIFICATIONS AND EXTENSIONS TO EXPERIMENTS

a. Name: Nasiru Muhammad

b. School and email address: School of Physiology, nsmaaji@gmail.com

c. Experiment to be modified / extended

		AESC NO	
Original AESC number	2015	11	51B
Other M&Es :			0

d. Project Title: Effects of Methanol Extract of *Moringa oleifera* Leaves on Fructose-Induced Metabolic Dysfunction in Growing Wistar Rats

		No.	Species
e.	Number and species of animals originally approved:	120	Wistar
f.	Number of additional animals previously allocated on M&Es:	0	
g.	Total number of animals allocated to the experiment to date:	0	
h.	Number of animals used to date:	0	

i. Specific modification / extension requested: To use the Sprague Dawley strain of rats for the study instead of Wistar rats.

j. Motivation for modification / extension: The Wistar is an outbred strain. Following discussions with the CAS, I have been informed that there may be long delays with the supply of the requisite numbers of animals to undertake the project. Previous studies have successfully used Sprague Dawley rats in high fructose diet-induced metabolic dysfunction studies (Huang et al 2004; Axelsen et al., 2010). The Sprague Dawley rats are thus an appropriate replacement strain for my study.
All other interventions will remain unchanged.

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Date: 02/02/2016 Signature: 

RECOMMENDATIONS: Approved. The use of S Dawley rats instead of Wistar rats for the study.

Date: 3 February 2016 Signature: 
Chairman, AESC