IN VIVO ANTI-OBESITY EFFECTS AND PHYTOCHEMICAL PROFILES OF DICHLOROMETHANE STEM BARK EXTRACTS OF *Piliostigma thonningii* (SCHUM) and *Lonchocarpus eriocalyx* (HARMS) IN MICE

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DECLARATION

I, Okioga Farida Moraa, confirm that this thesis is my original work and has not been submitted for any other award in any other university

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DEDICATION

I dedicated this work to God, family and my dear friends from whom I get my inspiration and passion.

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ABBREVIATIONS AND ACRONYMS

AC	Abdominal Circumference
AgRP	Agouti-Related Protein
AI	The Atherogenic index
ATP	Adenosine 5′-triphosphate,
BAI	Body Adiposity Index
BMI	Basal Metabolic Index
BW	Body weight
DCM	Dichloromethane
DMPA	Depo-medroxyprogesterone Acetate
DMSO	Dimethyl Sulphoxide
DNA	Deoxyribonucleic acid
FTO	Fat mass and obesity
IL-6	Interleukin-6
MRI	Magnetic Resonance Imaging
NA	Nasal-Anal length
NIST	National Institute of Standards and Technology
ANOVA	Analysis of variance
NPY	Neuropeptide-Y
OECD	Organization for Economic Corporation and Development
GLP-1	Glucagon-like peptide

ABSTRACT

Obesity is a chronic metabolic condition described as an imbalance between energy consumption and utilization in the body. There is no particular cause for obesity, though, different contributing features such as consumption of high-caloric diets, sedentary lifestyle, physical inactivity, gender, genetics, and metabolic predisposition are implicated. Considering the devastating sequelae of obesity, development of resistance to conventional drugs, lack of curative armamentaria, high costs of symptomatic and palliative drugs and their low efficacies and adverse effects, there is a need for alternative therapies. Despite the extensive ethnomedicinal usage of *Piliostigma thonningii* and *Lonchocarpus eriocalyx* to manage obesity and associated complications, there is insufficient empirical data to validate their healing claims. Thus, this research was carefully deliberated to investigate *in vivo* antiobesity effects of *P. thonningii* and *L. eriocalyx* DCM stem bark extracts at dosages of 75, 150 and 300 mg/Kgbw, in Depo-medroxyprogesterone Acetate (DMPA)-induced female obese mice. Additionally, quantitative phytochemistry of the two DCM extracts was performed by Gas Chromatography-Mass spectrometry. In this study, extract-treated DMPA-induced obese mice had significantly lower body weights, organ weights, abdominal circumferences, fat pad weights, body adiposity indices and body mass indices than those of the negative control mice and were generally comparable with those in the normal control mice, indicating significantly high anti-obesity efficacy. The atherogenic indices, lipid profiles and blood glucose levels of the extract-treated mice were considerably lower relative to those seen in obese control mice**,** while the non-atherogenic lipid profile (high-density lipoprotein cholesterol) was notably higher compared with the levels in the negative control mice, thus validating the efficacy in ameliorating obesity-associated complications. Notably, the anti-obesity effect of the two studied extracts showed a positive dose-dependent relationship. Generally, the effects of the two extracts on studied variables were non-significant at the same dosage. Anti-obesity efficacies of the two plant extracts were strongly attributed to various phyto-compounds that varied in concentrations, as shown by quantitative phytochemical analysis. Further studies to demystify the mechanisms of action of the two DCM extracts and those on other solvents, as well as using other experimental animals should be carried out to assess the full potential of the two plants. Additionally, bioactivity-guided isolation of the active phytochemicals and determination of their modes of anti-obesity action and toxicity profiles should be performed.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Obesity refers to a metabolic condition featured by an excessive deposition of body fats which may be exacerbated by various environmental, social, and genetic factors. However, obesity majorly results from an imbalance between energy surplus and its utilization during metabolism, often due to a sedentary lifestyle (Albaik *et al.*, 2016). Research has shown that obesity characterizes chronic oxidative stress, low state of chronic inflammation, and metabolic syndromes (Liu *et al.*, 2017). Excessive body adiposity can promote other long-term conditions such as osteoarthritis, diabetes mellitus, ischemic and hypertensive heart diseases, respiratory diseases as well as different types of cancers (Lustig *et al.*, 2022).

Globally, obesity prevalence has tripled since 1975. Moreover, the incidence of obesity is constantly growing worldwide, and it is approximated that 39% of the global population by 2030 will be overweight, 20% of which will be obese (Zielinska *et al.*, 2019; Chatterjee *et al.,* 2020). Recent evidence indicates that overweight and obesity are increasing in Sub-Saharan Africa, including Kenya, at a rate of 5% per year on average (Arika *et al.*, 2019). Thus, obesity-related medical expenses account for about 40% of the healthcare budget, posing considerable healthcare, economic, and societal challenges if not mitigated adequately (Rome, 2011).

Most conventional anti-obesity armamentaria, such as appetite suppressants (Lorcaserin), metabolic promoters, and drugs that block absorption and accumulation of lipids, such as Orlistat and phentermine, have demonstrated appreciable

symptomatic efficacy. However, many have failed to manage the condition adequately, coupled with adverse effects, leading to some withdrawal, like Sibutramine (Liu *et al.,* 2017). Therefore, alternative efficacious anti-obesity drugs, especially those derived from plants or their extracts, may affordably and feasibly help sustain weight loss (Rodgers *et al.,* 2012). Research indicates that plants like *Carthamus tinctorius* L, *Astragalus membranaceus,* and *Panax ginseng* possesses anti-obesity properties (Liu *et al.,* 2017). Moreover, medicinal plants exert their anti-obesity efficacy through several mechanisms like inhibiting lipogenesis, fat absorption, and modulation of appetite and metabolism (Payab *et al.,* 2018). Besides, phytoconstituents such as alkaloids, terpenes, saponins, and tannins are linked to reduced HDL, LDL, total cholesterol, and triglycerides due to their hypo-lipidemic effects (Zhang *et al.,* 2015).

Despite the potential of herbal medicine in managing obesity and medicinal plants being a bulwark of diverse efficacious amalgams, only a few have been explored empirically (Salmerón-Manzano *et al*., 2020). For instance, the anti-obesity efficacy of *Lonchocarpus eriocalyx* and *Piliostigma thonningii* haven't been scientifically studied. Thus, this study explored the *in vivo* anti-obesity effects, as well as quantitative phytochemical profiles of *Lonchocarpus eriocalyx* and *Piliostigma thonningii* DCM stem bark extracts as promising sources of anti-obesity therapies that are safe, efficacious, and affordable.

1.2 Statement of the problem

Obesity remains the 5th principal cause of mortality and morbidity globally. Besides, it causes multiple metabolic maladies such as cardiovascular illnesses, dementia, type 2 diabetes, sleep apnea, and stroke. In Kenya, obesity prevalence is estimated to be 25%,

with women being at a higher risk (74 %) than men (24 %) (Sigei *et al.,* 2018; Mohamed *et al.,* 2019)

Obesity is linked with reduced socioeconomic productivity, stigma, and high medication-related costs (Seidell and Halberstadt, 2015). Besides, the high cost of conventional medicines exerts a significant healthcare burden on the affected subjects, society, and the government. For instance, research has shown that obese individuals spend 32 % more on managing their condition and its associated comorbidities (Yusefzadeh *et al.,* 2019).

Conventional anti-obesity drugs have suffered various drawbacks, including dependency and abuse, adverse effects on the monoamine neurotransmitters, unavailability, and unaffordability for middle and low-income earners (Nwobodo, 2015; Qi, 2018; Dai *et al*., 2020). Surgery, which is opted for in severe cases, may result in complications such as hypertension, deep vein thrombosis, anemia, and post-surgical infections (Liu *et al.,* 2017).

1.3 Justification of the study

Plants rich in antioxidants are known to scavenge free radical oxygen species directly as well as fostering endogenous antioxidant defenses to avert oxidative stress, which is the main cause of obesity (Kaur *et al.,* 2014). Considering the unaffordability, low efficacy, and adverse effects of synthetic anti-obesity drugs prescribed conventionally, medicinal plants are viable alternatives due to their enormous pharmacologically active amalgams (Liu *et al.,* 2014). Additionally, the prominence of herbal products in managing obesity spans over 2000 years, albeit with insufficient

empirical validation of their efficacy and safety (Liu *et al.*, 2017).

Despite the widespread folkloric application of various parts of *P. thonningii* and *L. eriocalyx* to manage obesity and its associated disorders worldwide, their healing potential is yet to be validated empirically (Dasofunjo *et al.*, 2013; Noufou *et al.*, 2016; Ochung, 2016). Thus, this present study had been carefully considered to examine the anti-obesity efficacy and the phytochemical composition of *Piliostigma thonningii* and *Lonchocarpus eriocalyx* DCM stem bark extracts. This research valorized the studied plant extracts as possible sources of lead compounds for developing safe, efficacious, and affordable anti-obesity armamentaria upon further investigations.

1.3 Research questions

Does *Piliostigma thonningii* and *Lonchocarpus eriocalyx* DCM stem bark extracts*;*

i. Cause effects on anthropometric parameters following Depo-medroxyprogesterone Acetate (DMPA)- induced obesity in mice?
ii. Have effects on lipid profiles and blood glucose levels following DMPA-induced obesity in mice?
iii. Contain anti-obesity-associated phytochemicals?

1.4 Objectives

1.4.1 General objective

To determine *in vivo* anti-obesity effects and phytochemical composition of DCM extracts of *Piliostigma thonningii* and *Lonchocarpus eriocalyx*.

1.4.2 Specific objectives

i. To determine the effects of DCM stem bark extracts of *Piliostigma*

thonningii and *Lonchocarpus eriocalyx* on anthropometric parameters following DMPA-induced obesity in mice.

ii. To determine the effects of DCM stem bark extracts of *Piliostigma thonningii* and *Lonchocarpus eriocalyx* on lipid profiles and blood glucose levels following DMPA-induced obesity in mice.

iii. To determine the quantitative phytochemical profiles of *Piliostigma thonningii* and *Lonchocarpus eriocalyx* DCM stem bark extracts.

1.5 Study significance

The findings of this research will provide 'qualified leads' in the synthesis of new anti-obesity agents that are safe, affordable and effective.

CHAPTER TWO

LITERATURE REVIEW

2.1 Obesity

Obesity is a complex, largely preventable chronic condition that develops when excess fat is deposited in organs that participate in metabolic processes, such as adipose tissues, muscles, liver and pancreatic islets (Wiechert *et al.,* 2021). Obesity affects most body functions which results in the emergence of several non-communicable conditions like diabetes, musculoskeletal complications, especially osteoarthritis, cardiovascular diseases, primarily heart attacks, kidney disease and some cancers, including those of the breast, kidney, endometrium, colon, liver, ovary, prostate, and gallbladder (Rana *et al.,* 2021; Iftikhar *et al.,* 2022).

Glycerol and free fatty acids are esterified to form fats; excess is stored in adipocytes. During fasting, the adipocytes provide energy to the body by releasing free fatty acids and aerobically metabolized glycerol. The major components of adipose tissue are Brown adipose tissue (BAT) and White adipose tissue (WAT). BAT fat cells are less abundant of the two, with high mitochondria population. It is also highly vascularized and is implicated in adaptive thermogenesis, energy balance, and lipid oxidation. In contrast, WAT is the most abundant, least vascularized, and has few mitochondria. Notably, WAT tissue also has other cells, for example, fibroblasts, immune cells, endothelial and fibroblastic pre-adipocytes (Shalitin and Gat-Yablonsk, 2021). Moreover, WAT tissues secrete several endocrine hormones and their closely connected cytokines, which affect peripheral tissues and the central nervous network. Furthermore, it is connected to obesity pathogenesis and regulation (Lee *et al.,* 2014; Kumar *et al.,* 2022).

Obesity is a multifactorial illness caused by several parameters such as genetics, lifestyle, environment, and diet. Furthermore, this illness affects persons of all sex, ages, races and ethnicities (Jaca *et al.*, 2020). Imbalance in energy between calorie intake as well as its expenditure is the fundamental cause of obesity. Worldwide, the main risk factor for obesity is increased consumption of calorie-rich, refined and processed foods that are low in vitamins, minerals, as well as other micronutrients but are rich in fats, sugars, and salts; others include enteric infections, lack or minimal physical activity as facilitated by change in mode of transport, nature of many forms of jobs and increasing urbanization, nutrients supplements, environmental contaminants, and eating abnormalities for instance night eating syndrome and binge eating disorder. In Africa and particularly in Kenya, wealth and prestige are portrayed culturally by obese individuals (Onyango and Onyango, 2018; Edith, 2019).

Obesity cases have also increased by factors such as alcohol consumption, smoking and the use of some drugs (contraceptive, psychotropic drugs, steroid hormones, diabetic medications, and anti-hypertensive drugs). Most individuals eat due to anger, negative emotions, sadness, and boredom; hence a person's psychological state may also contribute to obesity. Body weight can increase due to insomnia (Arseneau *et al.*, 2019; Jaca *et al.*, 2020).

The past forty year, global increase in obesity have been witnessed among children across all ages, these, in turn, raises the risk of cardiovascular diseases, hypertension, early puberty in children, metabolic syndrome, high cholesterol levels and sleep disorders like obstructive sleep apnea (Shabana *et al*., 2020; Azmi *et al*., 2021). Besides, obese

adolescents and children may suffer from psychological problems, for instance, anxiety, depression, eating disorders, poor body image, poor peer relationships, and low self-esteem. In addition, there are high risks of obesity once the children develop into adulthood (Kansra *et al.,* 2021; Shalitin and Gat-Yablonsk, 2021). Furthermore, the body's process, such as metabolism, reduces as one gets older hence these persons require a couple of calories to maintain body weight. These individuals are predisposed to obesity when they consume excess caloric food, which is changed into triglycerides, the body fats that lead to weight gain (Shantaram *et al.*, 2017).

Gender predisposes people to obesity. Generally, during reproduction years (especially during pregnancy and postpartum), women gain weight (Chowdhury *et al.*, 2018). Also, rate of metabolism of postmenopausal women is reduced, and overconsumption of food at this point, partly clarifies why numerous women gain weight after menopause. On the other hand, men need more calories to maintain their body weight since they use more energy when resting than women (Shantaram *et al.*, 2017).

2.2 The pathophysiology and pathogenesis of obesity

Numerous hormones participate in the regulation and pathophysiology of obesity. Ghrelin acts peripherally by inducing appetite, an orexigenic peptide hormone formed from the stomach. Contrarily, Cholecystokinin, Glucagon-like peptide-1 and Peptide YY limit eating, promotes digestion, food absorption and inhibits overfeeding. They are gut-derived anorectic hormones (Aktar *et al.,* 2017).

Adipokines are produced and secreted by adipocytes, including adiponectin, interleukin-6, tumour necrosis factor-alpha, and leptin (Aktar *et al.,* 2017). WAT

produces and release leptin, and also regulates eating and energy use, thus helping to maintain lasting normal body weight. Leptin inhibits appetite by providing the sensation of satiety when it binds to the neurons that modulate appetite and specific receptors on the arcuate nucleus located in the hypothalamus (Shalitin and Gat-Yablonski, 2021). Moreover, leptin promotes thermogenesis. Therefore, deficiency of leptin results in lesser energy expenditure and is associated with obesity, although this is not true for every obese person, as some have raised levels of leptin (Kumar *et al.*, 2022).

Adiponectin is an adipokines released chiefly by differentiated adipocytes or derived from plasma proteins. Adiponectin is an insulin sensitizer and is involved in homeostasis. It has anti-atherogenic and anti-inflammatory activities. Levels of adiponectin are low in obese and diabetic persons, but following weight loss, their levels can return to normal (Shalitin and Gat-Yablonski, 2021, Kumar *et al.*, 2022).

Obese persons usually have elevated contents of TNF-α in serum, which favors systemic acute inflammatory-phase response where pro-inflammatory cytokine such as IL-6 are secreted while anti-inflammatory molecules like adiponectin is suppressed (Verdile *et al.*, 2015). Moreover, TNF-α raises the association of electrons with oxygen to produce superoxide radicals (Tang *et al*., 2021). On the contrary, cytokine IL-6 is produced by numerous body cells, namely monocytes, endothelial cells, β-pancreatic cells, adipocytes, and macrophages. Its roles include regulation of energy homeostasis, inflammation and prompting the transition of inflammatory illness from acute to chronic. Higher levels of serum IL-6 in humans are linked to diabetes mellitus, obesity and elevated blood pressure (Kumar *et al.*, 2022).

Agouti-related protein (AgRP) neurons produce endocrine factors; Neuropeptide-Y (NPY), gamma-aminobutyric acid and AgRP protein, which is activated during fasting and promotes feeding (Nonogaki, 2022). AgRP reduces leptin response while enhancing insulin activity which restrains food consumption and generates NPY in the arcuate nucleus, therefore, hyperphagic behavior is brought about by aberrant stimulation of AgRP neurons (Kelleni, 2018; Nonogaki, 2022). Contrary, NPY promotes feeding and increases anabolism while decreasing catabolism. α-melanocortical stimulating hormone is secreted by pro-opiomelanocortin neurons also located in the arcuate nucleus, it in turn, attaches to melanocortin-4 receptor and stimulates satiety by suppressing feeding, besides it also stimulates anorexia (Kumar *et al.*, 2022).

The para branchial nucleus around the superior cerebellar peduncle has calcitonin gene-related protein neurons that cause potentially fatal anorexia. Single-gene mutations are one of the causes of obesity. The mutation can occur in genes encoding melano-cortin-4 receptor, dopamine receptor D4, and leptin proteins. Fat mass and obesity (FTO) linked with gene encoding the FTO protein (alpha-ketoglutarate dependent dioxygenase) is produced in the hypothalamus and adipocytes (Pan *et al*., 2018). Also, a single nucleotide mutation in the 1st intron for the FTO gene may lead to obesity. In unusual circumstances, hormonal imbalance, infections, tumor, severe traumatic events, or hypothalamic lesions predispose some people to the risk of obesity (Kumar *et al*., 2022). The polycystic ovarian syndrome affects women, while Cushing syndrome, hypogonadism and hypothyroidism affect men (Shantaram *et al.*, 2017).

2.3 Types of obesity

Based on body fat distribution, two distinctive categories of obesity are known; android and gynoid obesity (Purnell, 2018; Xia *et al.,* 2022). Android obesity, also denoted as apple-shaped obesity or abdominal obesity, is more prevalent in men but is present in some women. It occurs when body fats are deposited mostly in the upper body, specifically the abdominal area. In puberty, presumably due to higher testosterone levels, boys develop an apple-shaped body. Android obesity is linked to type 2 diabetes mellitus and heart disease in both men and women (Purnell, 2018; Xia *et al.,* 2022).

Gynoid/pear-shaped obesity which is more prevalent among women, develops when fat accumulates in the femoral and gluteal parts of the lower body. In puberty, increased estrogens cause pear shape body in women. Gynoid obesity has a lower risk of causing metabolic and cardiovascular illnesses (Klaver *et al.,* 2018; Xia *et al.,* 2022). Genetics or epigenetics could determine fat distribution, involving other factors like metabolomics, diet, lifestyle of individuals, and microbiomics (Xia *et al.,* 2022).

2.4 Epidemiological impacts of obesity

In the past, obesity was viewed as a developed economies medical problem; however, lately its prevalence has been escalating in developing nations and moderately rich economies (Balakumar *et al.,* 2016). Obesity is more common among middle-aged grown-ups in developing states, specifically women of reproductive age between fifteen and forty-nine years. In developed nations individuals of both genders and all ages are affected (Seidell and Halberstadt, 2015).

Globally, in 2016 more than one point nine billion adults were overweight, with more than six hundred and fifty million obese. Global reports showed that between 2016 and 2018, approximately forty million kids under five years as well as three hundred and forty million adolescents were obese. In 2018, roughly nine point five million children under five years suffered from obesity or overweight in Africa (Gouda *et al*., 2019; WHO, 2022). If the present-day trend persists, it is projected globally that by the year 2030, twenty percent of the grown-up populace will be obese and thirty-eight percent will be overweight (Nittari *et al.,* 2020). Furthermore, the universal COVID-19 pandemic that caused confinement leading to forced change in lifestyle behaviour encouraged an obesogenic environment (Wiechert and Holzapfel 2021).

Overweight and obese people face both social and psychological stigmata due to discrimination in their personal and work environment. Most of these individuals end up depressed and have low self-esteem (Purnell, 2018). An obese person also burdens health care expenses of not less than 25% more than a healthy individual, according to research conducted in different countries. Also, obesity has led to premature loss of life, low work productivity, and increased disability cases, thus generating additional economic costs. Approximately eight percent of all worldwide mortality and disability-adjusted life are caused by obesity (Bollapragada *et al.*, 2017; Gil-Rojas *et al.,* 2019; CMAJ, 2020).

2.5 Diagnosis of obesity

The 1st stage in obesity care is obtaining a comprehensive history of the patient suspected to have obesity. This allows the specialist to determine the previous cases of childhood or adolescent obesity. Others carried out tests that may impair treatment

include diet, physical activity, psychological health problems as well as eating disorders. Additionally, comprehensive assessment enables the physician to design treatment schemes tailored to the client's goals (Tchang *et al.,* 2021).

Diagnosis for obesity is based on physical body examination such as waist-to-hip ratio, body mass index (BMI), and impact of comorbid diseases, including sleep disorders, diabetes, hypertension, dyslipidaemia, respiratory, joint, and cardiovascular diseases. In general, clinical practice BMI usually assesses body fat due to it being inexpensive and simple. The BMI index is given by individual body weight (kg) divided by squared height (m^2). Adult people with BMI greater or equal to 25.0 kg/m^2 are classified as overweight. Individuals whose BMI ranges from 25.0-29.9 kg/m^2 are pre-obese. According to BMI ranges, obese people are divided into class I, class II, and class III. Class I obese people have BMIs ranging between 30.0 to 34.9 kg/m2, class II obese people have BMIs ranging between 35.0 to 39.9 kg/m2, whereas class III/extreme obese people have BMIs of 40.0 kg/m2 or more (Aktar *et al.*, 2017).

The abdominal adiposity' amount is assessed using waist-to-hip ratio, which greatly compares to intra-abdominal fat content. Waist circumference is obtained using a tape measure, where both waist and hip measurements are obtained and divided to obtain the ratio. In order to identify individuals with increased visceral adiposity, regular waist circumference measurement is recommended. Adiposity-related health risks such as insulin resistance and production of pro-inflammatory cytokines are fueled by escalating visceral adiposity (Tang *et al*., 2021). Men and women with waist circumference above 102 and 88cm, respectively, are classified as obese and are at a greater risk of developing co-maladies, for instance, diabetes and heart diseases

(Purnell, 2018; CMAJ, 2020).

Fasting blood glucose levels, uric acid levels, thyroid efficiency, serum lipid profile, liver function biomarkers, liver biopsy and ultrasound are examples of laboratory examinations that are carried out to ascertain obesity and its comorbidity illnesses. Positive cardiovascular tests imply more endocrine testing to determine Cushing's syndrome or hypothalamic disease, while abnormal liver function tests indicate non-alcoholic fatty liver ailments or other liver related disorders (Aktar *et al.*, 2017).

Fat mass assessment is done by imaging techniques; computed tomography scanning, dual-energy x-ray absorptiometry and magnetic resonance imaging and. These imaging methods are cost-prohibitive and impractical; hence they are mostly utilized in research (Buch *et al*., 2022). Indirect measurement of fat mass is by underwater weighing, bioimpedance analysis (BIA), and air displacement. Fitness centres and clinics operated by obesity medicinal specialists offer BIA and air displacement (they assess body fat composition and adipose tissue depots). However, the general use of BIA and air displacement in the care of obese and overweight patients are still not recommended (Purnell, 2018).

2.6 Obesity and oxidative stress

Biological systems' damage could be due to oxidative adversity, which is the dysregulation between the production and accumulation of reactive radicals derived from nitrogen and oxygen (Liguori *et al*., 2018). Free radicals such as nitrogen species (RNS) and reactive oxygen species (ROS) consist of superoxide, hydroxyl and nitric oxide radicals. Besides, hydrogen peroxide and peroxynitrite are other non-free oxidative species. ROS are mainly generated in the mitochondria when electrons leak through

the respiratory chain (Ozcan and Ogun, 2015). Enzyme in the endoplasmic reticulum, lysosomes, peroxisomes, and cytosol produce ROS. The beneficial aspects of ROS/RNS are exhibited in low concentrations, including defense against pathogenic microbes, a process facilitated by the immune system as well as intracellular signaling. On the contrary, ROS/RNS are injurious at a high concentration as they may destroy proteins, DNA and lipids, resulting in cell mortality and tissue injury (Pizzino *et al.,* 2017; Gutiérrez-López *et al.,* 2021).

Excess fat is deposited in various parts of an obese individual's body. This may result in an abnormal rise in fatty acid levels in serum, which sequentially damages glucose metabolism. It also favors the accumulation of energy substrate (glucose and fats) in muscles, adipose tissue, and liver and encourages increased oxidation in the peroxisome and mitochondria (Ozcan and Ogun, 2015). Consequently, excess electrons are supplied to the respiratory chain, leading to more ATP production. Still due to minimal or lack of physical activity in obese individual, which translates to low respiration rate, the high proton motive force results in mitochondrial superoxide formation and oxidative stress (Ozcan and Ogun, 2015).

Superoxide species are mainly generated by NADPH oxidases and to a less extend by metabolic enzymes for instant xanthine oxidoreductase, lipoxygenase, cytochrome p450, and cyclooxygenase one and two as one of its by-products. Owing to its anionic nature, superoxide species diffuse into the cell via the biological membranes, which are successively reduced to form hydroxyl radical and hydrogen peroxide (Sharifi-Rad *et al.,* 2020). Furthermore, additional types of ROS are formed, namely hypochlorite ions, alkoxyl and peroxyl species. Cumulatively all these ROS can be

very detrimental to cells, where they deactivate several processes, ultimately triggering irreversible cell apoptosis or necrosis (Sharifi-Rad *et al.,* 2020).

2.7 Interventions for obesity and oxidative damage

2.7.1 Conventional management

The foundation of obesity management is lifestyle modification. Antioxidant-rich food with low calories plays a critical role in providing the cell's energy and boost the immune system by scavenging ROS (Israel Pérez-Torres *et al*., 2021). Increased physical activity increases energy expenditure that may lead to weight loss. Despite these efforts, an obese individual may be unable to decrease weight, consequently, anti-obesity drug therapy may be an option (Bollapragada *et al*., 2017).

Orlistat suppresses pancreatic and/or gastric lipases which decreases dietary fat absorption by 30%. Orlistat is saturated derivative of Lipastatin, which is isolated from *Streptomyces toxytricini.* Orlistat also reduces LDL and cholesterol levels, promotes glycemic regulation, and significantly decreases blood pressure in diabetic patients (Liu *et al.,* 2014; Qi, 2018). An experiment conducted on Orlistat weight loss activities showed that administration of one hundred and twenty milligrams of Orlistat 3 daily for one year decreased the chances of regaining weight. Fat-soluble vitamin deficiencies, steatorrhea, abdominal cramping, fecal urgency and incontinence, and liquid stool are some side-effects linked to this drug (Aktar *et al.,* 2017).

Sibutramine is a drug that reduces weight and effectively maintains weight loss through reduced appetite and increased satiety. This drug was developed initially as an anti-depressant and further research on its other effects discovered potent weight loss properties. Sibutramine inhibits the reuptake of norepinephrine and serotonin

without stimulating their discharge (Sombra and Anastasopoulou, 2022). This drug cannot be co-administered with monoamine oxidase inhibitors as well as selective serotonin reuptake inhibitors. The adverse effects of this drug are insomnia, increased blood pressure, nervousness and tachycardia are some of the adverse side effects associated with this drug. Unique side effects reported in some patients include constipation and dry mouth. (Aktar *et al.,* 2017).

Lorcaserin acts as a serotonin type-2C receptor agonist; hence, it has hypophagic properties. For a 5% weight loss goal, 10mg of lorcaserin is recommended two times daily for 12 weeks, as specified in the product license. Discontinuation of this drug is recommended if the target is not achieved (Sombra and Anastasopoulou, 2022). Headache, nausea, dizziness, somnolence, blurred vision, and gastrointestinal disturbance are some of the general side effects experienced after ingesting Lorcaserin (Aktar *et al.*, 2017).

Phentermine, an analogue of amphetamine, reduces appetite as a norepinephrine agonist in the central nervous system (CNS). The synergistic combination of this drug and topiramate enhances efficacy with lower toxicity. Topiramate, an anticonvulsant drug, is a promising anti-obesity agent owing to weight loss noted in epileptic patients using the drug (Sombra and Anastasopoulou, 2022). For a five percent weight loss goal, a dose of 46 mg topiramate and 7.5 mg phentermine are often prescribed daily for twelve weeks. If the goal is not attained, drug discontinuation is advised. Sleep disturbances, dry mouth, palpitations, constipation, metabolic acidosis, paraesthesia, dysgeusia, hypokalemia, alopecia, headache, attention deficits, and renal calculi are side effects associated with the medication. (Nicolucci and Maffeis, 2022).

The Bupropion/naltrexone combination is speculated to possess anorectic activity. The two are centrally acting medications where bupropion acts as a non-specific inhibitor of dopamine and norepinephrine transporters and is employed in treating depression and aids in smoking cessation (Sombra and Anastasopoulou, 2022). Naltrexone, on the other hand, is mainly employed in treating alcohol and opiate dependence syndrome. Naltrexone/bupropion combination triggers arcuate pro-opiomelanocortin neurons secrete a melanocyte stimulating hormone; an anorectic feeding signaling neuropeptide that venture to hypothalamic regions that controls feeding and body weight. To achieve a five percent weight loss goal, a dosage of 180 mg bupropion/16 mg naltrexone is administered twice daily for 12 weeks. Discontinuation of the drug is recommended if this goal is not achieved or when these side-effects are experienced; dizziness, vomiting, nausea, headache, and insomnia (Aktar *et al.,* 2017).

Liraglutide acts as GLP-1 receptor agonist that hinders rapid metabolism via dipeptidyl peptidase-IV. This injectable drug reduces glucagon response by stimulating the release of glucose-induced insulin, it also enables gastric emptying and suppresses appetite (Sombra and Anastasopoulou, 2022). Success has been achieved with its introduction to type 2 diabetic patients. To achieve a five percent weight loss goal for twelve weeks, a dosage of 1.2 to 1.8 mg is administered once day. If the drug is not effective, it should be discontinued. Liraglutide is usually well tolerated with the following adverse effect; nausea and vomiting (Kumar *et al.,* 2022).

Bariatric surgery is only suggested for class III patients or those with comorbid

diseases, for example, type 2 diabetes mellitus or cardiopulmonary disease with BMI $\geq 35 kg/m^2$ (Fruh, 2017; Gadde *et al*., 2018). Malabsorptive and restrictive methods are procedures employed in weight loss through surgery. They are achieved through vertical banded gastroplasty surgery, Roux-en-Y gastric bypass surgery and laparoscopic adjustable gastric banding (Lin and Li, 2021; Wiechert and Holzapfel 2021).

To maintain RNS and/or ROS at normal levels, tissues have various antioxidants that interact to lessen free radical cytotoxicity. Various natural antioxidant components are found within the body. They include compounds such as urate, ubiquinone, thioredoxin, and glutathione. Many proteins also have antioxidant activities, for example, lactoferrin, ferritin, Transferrin, ferritin, and caeruloplasmin. These proteins hijack and bind transition metals that probably initiate oxidative reactions (Israel Pérez-Torres *et al*., 2021). In addition, there are various antioxidant enzymes namely Ubiquinone oxidoreductase, glutathione reductase, superoxide dismutase, thioredoxin reductase, catalase, peroxiredoxins, glutathione S-transferase, and glutathione peroxidase (Cantó *et al*., 2015).

Moreover, paraoxonases or aryl dialkyl phosphatases are unique types of antioxidant enzymes that hydrolyze organophosphorus complexes, for instance, diazoxone or paraoxon insecticides. Esterase or paraoxonase-1 is an antioxidant enzyme that exhibits an atheroprotective effect through its association with HDL enzyme, which hydrolyzes oxidized LDL-cholesterol and is produced in the liver (Bordalo, 2018; Azmi *et al*., 2021). Esterase activities decrease in the following illnesses: diabetes mellitus, hypercholesterolemia, chronic renal failure, and metabolic syndrome.

Another unique antioxidant enzyme, heme oxygenase-1 decreases inflammation and oxidative stress. In addition, it is a rate-limiting enzyme in heme metabolism (Pérez-Torres *et al.*, 2021).

Drug –induced obesity in rodents for research

2.7.2 Herbal management

Since ancient times herbal medicines have been applied to diagnose, prevent, and treat innumerable diseases. About 80% of the people in the third world rely entirely on plants as their medicine sources due to their affordability, availability, and their effectiveness as a source of new therapeutic agents (Piero, *et al.*, 2015). Various plant extracts have anti-obesity properties and some which have been evaluated to manage obesity include herbs such as *Nigella sativa*, *Camellia sinensis, H. gordonii* (Basu *et al.*, 2011), methanolic leaf extracts from *Amaranthus dubious, Vigna unguiculata,* and *Curcubita pepo* (Nderitu *et al.*, 2017) *Nelumbo nucifera* leaf extracts, *Juniperus communis* (bark) and *Illicium religiosum* inhibits lipid peroxidation in liver and plasma (Arika *et al.*, 2019a). These few extracts are prospective medicaments when it comes to the management and treatment of increased body weight.

Moreover, when a diet is supplemented with natural plants, it results in weight loss and improved health. These plants have various constituents that scavenge ROS. Medicinal plant formulations are known to exhibit fewer adverse effects when compared to synthetic drugs, hence employed in managing body weight. Additionally, they are a major source of active constituent: catechins, polyphenol, carotenoids, capsaicinoids, flavonoids, isoflavones, and capsinoids (curcumin, resveratrol, quercetin, phloretin, and ferulic acid) (Jaradat *et al*., 2022; Yen *et al*., 2020). According to Marseglia *et al.* (2014), β-Carotene, and vitamin A, C and E, are plant

compounds that can reduce intracellular oxidative stress in the adipose tissue. This study explored the anti-obesity potential of *Lonchocarpus eriocalyx* and *Piliostigma thonningii* and validated their traditional use.

2.8 *Piliostigma thonningii* (Schumach.)

2.8.1 Plant profile

Piliostigma thonningii (Schumach.) belongs to the sub-family Caesalpinioideae and family Fabaceae and is a native of tropical Africa. Some of its names include Rhodesian bauhinia, wild bauhinia, camel's foot (English), *mchikichi, mchekeche,* (Swahili) *mutukutu* (Shona), *ihabahaba (Ndebele), khufaj jamal, tambareib, abu khameira, kharub* (Arabic), *kigali* (Luganda) and *mukura* (Embu) (Orwa *et al*., 2009).

It is an evergreen, deciduous, unisexual tree that grows up to four to fifteen meters in height with a rounded crown bearing a short, crooked bole. Its twigs are hairy, the hair is rusty in color, it also has a rough bark with longitudinal fissures, and when fresh, the bark appears creamy-brown but changes to grey-brown when older, and its roots are deep. Its green leaves are heavily veined, bi-lobed, and leathery in texture. It also has rusty-colored hair at the bottom part. The flowers are drooping with five white to pink petals. The fruit is hard and hairy, while its pod is flat and often split when fully mature. Several components can be extracted from the fruit pulp of *P. thonningii* example, starch, organic acids, fibre, proteins, vitamins, calcium oxalates and essential oils (Orwa *et al.,* 2009).

2.8.2 Therapeutic uses

The Embu community uses the bark decoction of *P. thonningii* to treat blood pressure (Kareru *et al.,* 2008). The Maasai community eats and chew leaves of *P. thoningii* to relieve thirst. Flavonoids, anthraquinones, saponins, tannins, steroids, alkaloids,

volatile oils and terpenoids are some of the active compounds isolated from diverse sections of *P. thonningii* plant and are employed in treating various diseases; stomachache, fever, skin infections, chronic ulcers, snake bite, hookworm, toothache, dysentery, respiratory ailments (chest pains, bronchitis and cough) and wound curing (Kwaji *et al*., 2010; Afolayan *et al*., 2018). Roots of *P. thonningii*'s are utilized in the treatment of STDs, prolonged menstruation, hemorrhage, and miscarriage (Hailemariam *et al.,* 2021).

Research on the ethanol extracts of *P. thonningii* pod has established antimicrobial effects against *Mycobacterium smegmatis, Candida krusei* and *Pseudomonas aeruginosa* (Makosa *et al.*, 2021). Olela *et al.* (2021) established that MeOH and aqueous extracts *P. thonningii* stem bark have anti-phlogostic and antinociceptive activities.

Figure 2.8.1: A photograph of *Piliostigma thoningii* (Schumach) taken in situ in Embu County, Kenya. (Captured by author on 24th August 2019)

2.9 *Lonchocarpus eriocalyx* (Harms)

2.9.1 Plant profile

Lonchocarpus eriocalyx (Harms) belongs to the family Fabaceae (leguminosae) and grows at an altitude of 500m-1680m. Although it is native to South America, it also grows naturally globally in Kenya, Benin, Niger, Tanzania, and Zambia. In Kenya, *L. eriocalyx* is commonly found Kerio valley, Mbeere, Marsabit, Tharaka Nithi, Elgeyo Marakwet, and in the lower eastern parts of Machakos. *Ilteroi* (samburu), *kinguuthe* (Kamba), *churutwa* (pokot) and *Muthigiriri* (Mbeere) are some of its

common names (Mubiu *et al.,* 2017).

It is a slender, deciduous tree with a height of about 15m. Its bark generally has a pale grey color and is smooth, in older trees, the bark flecks. Resinous exudate is produced when the tree is cut. The leaves length ranges from 3-6 cm and have the shape of an egg, a round apex, and a symmetric base. The leaf, when young, has hair on both the top and bottom parts, with the bottom part hair being whitish and woolly. The veins and midrib are also hairy. Flowers are erect and attractive in their pale violet color. The seed pod, with an area of 7cm by 3 cm is flattened and possesses a creamy brown color (Kokwaro, 2009).

2.9.2 Therapeutic uses

Medicinally, its bark decoction is employed by the Mbeere and Embu people to treat diabetes, rheumatism, and hypertension (Gachanja *et al.,* 2007). Atieno *et al.* (2020) reported that erpenoids; friedelin, stigmasterol glucoside, lupeol and stigmasterol were derived from *L. eriocalyx* leaf ethyl acetate extract. They also ascertained the analgesic effects of *L. eriocalyx* DCM and ethyl acetate leaf extracts.

In another study by Onyango *et al.,* (2018) lupeol, chrysin, friedelin, stigmasterol, lupenone, stigmasterol 3-*O*-β-glucoside, 3,5,7,2,4-pentahydroxyflavonol and apigenin were the eight compounds isolated from CH_2Cl_2 and MeOH leaf extracts. In addition, Mubui *et al.* (2017) demonstrated the antimicrobial activities of aqueous, methanol and chloroform extracts of *L. eriocalyx* root. The root bark of *L. eriocalyx* has been used to treat pimples and manage weight loss in Tanzania (Mubiu *et al.*, 2017). These extracts also demonstrated antibacterial effects on *Pseudomonas aeruginosa* and *Staphylococcus aureus* as well as antifungal properties on *Saccharomyces cerevisiae.* Even though several studies on plant-based anti-obesity

extracts exist, no study has been done on DCM stem extracts of *Piliostigma thonningii* and *Lonchocarpus eriocalyx* in relation to anti-obesity effects and quantitative phytochemical profiles. Thus, this study aims to quantitatively determine the phytochemical profiles and validate the anti-obesity properties of DCM stem extracts of *L. eriocalyx* and *P. thonningii* following DMPA-induced obesity mice.

Figure 2.8.2: A photograph of *Lonchocarpus eriocalyx* taken in situ in Embu County, Kenya. (Captured by Author on 24th August 2019).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant samples collection and preparation

Fresh stem barks were sparingly obtained from randomly selected mature study plants (*Piliostigma thonningii* and *Lonchocarpus eriocalyx)* in their natural habitation in Cianyi village, Mbeere North sub-county, Embu County in Kenya. A local herbalist assisted in identifying the plants, and they were chosen for this study based on their ethnomedicinal use as all-natural anti-obesity therapies. A capable botanist from Kenyatta University's Department of Plant Sciences collected, prepared, and taxonomically authenticated the voucher specimen number as *Piliostigma thonningii* GM001/2017 and *Lonchocarpus eriocalyx* GM002/2017 which were then stored in the university herbarium for future reference.

The collected plants were then put in woven sisal sacks and taken to the laboratory at Kenyatta University, where the study was conducted. They were spread evenly to dry on a flat laboratory bench at room temperature, away from direct sunlight, for 14 days after being cut into smaller pieces. An electric plant mill was used to grind the dried materials into a powder, then transferred to khaki envelopes with clear labels and preserved on a tidy, dry shelf in the lab in readiness for extraction.

3.2 Extraction

The extraction procedure of Harborne (1998) was adopted in this study with a few adjustments by Arika *et al*. (2019). The powdered materials of the study plants were weighed (200 g) and macerated separately in 750 ml of analytical grade dichloromethane (DCM) inside a conical flask closed tightly using aluminum foil. The mixtures were shaken periodically and then allowed to interact for 2 days, after

which, the decanted solution was filtered using Whatman grade 1 filter papers into clean conical flasks. After that, the rotary evaporator was used to concentrate the filtrates *in vacuo*, whearas the temperature in the water bath was maintained at 28 °C. Then the plant extracts were put in sterile, dry and pre-weighed glass containers, then allowed to dry completely at 25±2 °C for five days. After that, they were reweighed, and percentage yields were calculated according to a previously described equation (Truong *et al*., 2019) and recorded.

$$Percentage = \frac{Extract\ weight}{Weight\ of\ the\ soaked\ material}$$

3.3 Investigation of anti-obesity effects of *Piliostigma thonningii and Lonchocarpus eriocalyx* DCM extracts in mice

3.3.1 Animal model

Sixty healthy female Swiss albino mice weighing 20 to 25 g and aged between 4-6 weeks, were bred and housed in groups of five at Kenyatta University Animal Breeding and Experimentation facility found in the Department of Biochemistry, Microbiology, and Biotechnology. The experimental animals were maintained in routine conditions of 23± 2 °C and 65 % humidity, with exposure to 12 hour Sday/night cycle, in polypropylene cages that were fitted with bedding made of softwood shavings. They received clean drinking water and specially formulated rodent pellets *ad* libitum. Prior to the experiment, the animals had a 72-hour acclimatization period. After the experiment, the mice were disposed off, conforming to ethical procedures. The National Commission for Science, Technology, and Innovation provided the permit for this study (NACOSTI/P/22/22702).

3.3.2 Preparation of experimental drug doses

Three experimental doses; 75, 150 and 300 mg/Kilogram body weight of *P. thonningii* and *L. eriocalyx* DCM extracts, 10 mg/Kg bw of Orlistat (Reference drug) and

10mg/Kgbw of Depo-medroxyprogesterone Acetate (DMPA) were purposefully selected following a prior pilot study. These doses were prepared according to procedures of the Organisation for Economic Corporation and Development (OECD, 2008) (Erhierhie *et al*., 2014).

$$\text{Adminstration dose } \left(\frac{\text{mg}}{\text{Kgbw}}\right) = \frac{animal\ body\ weight\ (g)}{1000\ g} X\ selected\ dose$$

Accordingly, the extract weighing 7.5 mg was dissolved in 2.5 ml 1% of dimethyl sulphoxide (DMSO) to yield a dose of 300 mg/Kgbw, appropriate for a mouse weighing averagely 25 g. After that, enough volume for five mice receiving this dose was prepared by multiplying by a factor of seven, doubled, and serially diluted two-fold using 1 % DMSO to obtain 150 mg/Kgbw, and 75 mg/Kgbw of the studied extracts. The same method was used in preparing 10 mg/Kgbw of Orlistat and 10 mg/Kgbw of DMPA for the administration to the positive control group mice and induction of obesity.

3.3.3 Induction of obesity

In this study, 10 mg/Kgbw of DMPA was administered subcutaneously into the experimental mice daily for 28 days to induce obesity. DMPA is known to induce hyperphagia in mice (Nderitu *et al*., 2017).

3.3.4 Experimental design

This research adopted a completely randomized research design. The animals in this study were selected randomly and then allocated into 6 groups (Group I to VI), each comprising 5 animals. The normal control group mice (Group I) were administered 1% DMSO orally and injection of 1 % DMSO subcutaneously after 30 minutes. The negative control group mice (Group II) received 1 % DMSO orally and 10 mg/Kgbw of DMPA subcutaneously after 30 minutes. The positive control mice (Group III) were administered 10 mg/Kgbw of Orlistat (the reference drug) orally and

10 mg/Kgbw of DMPA subcutaneously after 30 minutes. The extract-treated mice (Groups IV-VI) were orally administered with extracts of *P. thonningii* or *L. eriocalyx* dose levels of 75, 150 and 300 mg/Kgbw and then injected 10 mg/Kgbw of DMPA after 30 minutes. All treatments were administered once every day for 28 days. Table 3.1 shows the design layout.

Table 3.1: Experimental design

Experimental Group	Treatment
Group I	1% DMSO (*p.o*) + 1% DMSO (*s.c*)
Group II	1% DMSO (*p.o*) + DMPA (10 mg/Kg BW; *s.c*)
Group III	Orlistat (10 mg/Kgbw; *p.o*) + DMPA (10 mg/Kgbw; *s.c*)
Group IV	Extract (75 mg/Kgbw; *p.o*) + DMPA (10 mg/Kgbw; *s.c*)
Group V	Extract (150 mg/Kgbw; *p.o*) + DMPA (10 mg/Kgbw; *s.c*)
Group VI	Extract (300 mg/Kgbw; *p.o*) + DMPA (10 mg/Kgbw; *s.c*)

Each Group comprised five mice; Group I: Normal Control mice; Group II: Negative Control mice; Group III: Positive Control mice; Groups IV-VI: Extract-Treated mice; DMSO: Dimethyl sulphoxide; DMPA: Depo-medroxyprogesterone Acetate; *p.o: Per os* (Oral); *s.c:* Subcutaneous; Extract: DCM stem bark extract of either *P. thonningii* or *L. eriocalyx.*

3.3.5 Determination of anthropometric measures of obesity

Anthropometric parameters, which characterize obesity, including weekly body weight, fat pad weights, Abdominal circumference (AC), Body mass Index (BMI), nasal -Anal length (NA), and the body adiposity index (BAI) of the experimental mice were determined following procedures reported by Arika *et al*. (2019) and Nderitu *et al*. (2017). The weekly body weights of each experimental mouse were measured using an analytical digital weighing scale and recorded accordingly. Measuring the largest area of the experimental mouse's abdomen, which was held in ventral position, using an inextensible calibrated thread, with a 0.1 cm accuracy, provided the abdominal circumference in animals. In this study, BMI was determined based on a previously described formula (Taylor and Phillips, 1996) as follows;

$$\text{BMI} = \frac{\text{Body weight (g)}}{\text{Body length (cm}^2\text{)}}$$

After that, the BMI of each experimental mouse was appraised based on the Lee index (Lee and Technical Assistance of Elizabeth Clark, 1929). Then, the weekly change in BMI for each experimental mouse was computed and tabulated.

3.3.6 Determination of adipose depots and body adiposity index

The experimental animals were euthanized and subjected to celiotomy to determine the adipose depots in following protocols reported by Arika *et al*. (2019). In the current study, an incision was made along the sagittal midline plane and the intestines were dissected to reveal the retroperitoneum. The retroperitoneal fat pad was removed horizontally beneath the kidneys, whereas the perirenal fat pad was excised atop.

Parametrial fat pads were dissected along the mid-base of uterus through to the extremities of the ovaries. Also, the mesenteric fat pad was obtained by gently pulling it from the duodenum along the small intestines through to the colon. After that, the inguinal subcutaneous fat pad was exposed and excised out of the underlying muscle, and brown adipose tissue was removed from the interscapular region of the mouse's neck. Thereafter, all the dissected fat pads were accurately weighed using an analytical digital scale and recorded in grams. The body adiposity index (BAI) in this study was computed according to the previously described formula (Leopoldo *et al*., 2016) and expressed as a percentage as follows;

$$\text{BAI (\%)} = \frac{\text{Fat pad weights (MS + RP + PT + PR + SC + BAT) g}}{\text{Final body weight (g)}} \times 100$$

Where, BAI: Body adiposity index; MS: Mesenteric; RP: Retroperitoneal; PT: Parametrial; PR; Perirenal; SC: Subcutaneous; BAT: Brown Adipose Tissue.

3.3.7 Determination of relative organ weights

The procedure utilized by Arika *et al.* (2019) was adopted to determine the liver, lungs, spleen, heart, brain and kidney's relative organ weights of experimental mice. The individual weights of these selected organs were accurately measured using an analytical digital scale and applied in computing the relative organ weights as follows;

$$\text{Relative organ weight (\%)} = \frac{\text{Organ weights}}{\text{Animal body weight (g)}} \times 100$$

3.3.8 Determination of random blood glucose level, lipid profile, and atherogenic index

Following euthanasia, fresh blood was collected through cardiac puncture of each experimental mouse using a 23-gauge needle fitted into a 5 ml syringe and transferred carefully in a plain vacutainer. After that, a drop of the collected blood samples was loaded on a glucose strip fitted into a Hypoguard glucose analyzer (Woodbridge, England) to blood glucose levels.

The standard operating procedures of the Department of Laboratory Medicine, Thika Level 5 Hospital were adopted in lipid profile assay. The blood samples were centrifugated at 2400 revolutions per minute for ten minutes using a top-bench centrifuge and then serum was aspirated and transferred into sterilized Eppendorf tubes. The concentrations of total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were analysed by an analytical biochemistry auto-analyzer (Olympus model). The concentration of very-low-density lipoprotein cholesterol (VLDL-C) was computed by btaining the triglyceride concentration and dividing it by five, as reported by Arika *et al.* (2019). Plasma atherogenic index (AI) was computed using the formula given

by Zalejska-Fiolka *et al*. (2019) as follows;

$$\text{Atherogenic Index (AI)} = \text{Log}\left(\frac{\text{TG}}{\text{HDL} - \text{C}}\right)$$

3.4 Quantitative phytochemistry of *Piliostigma thonningii and Lonchocarpus eriocalyx* DCM extracts

Based on a previously established approach, gas chromatography and mass spectrometry (GC-MS) was used to undertake quantitative phytochemical analysis of the two plant extracts (Babatunde *et al*., 2016; Ibrahim *et al*., 2021).

3.4.1 Preparation of plant samples for GC-MS analysis

The DCM extracts *P. thonningii* and *L. eriocalyx* were accurately weighed (1 mg) resuspended in 1 ml of analytical grade DCM in Eppendorf tubes and vortexed for 30 seconds. The samples were let to stand at 25±2 ºC for 24 hours. Thereafter, they were sonicated for 20 minutes and then centrifugated at 1400 revolutions per minute for 5 minutes. The resultant supernatants were carefully aspirated into clean, sterilised glass vials and dried in a desiccator containing anhydrous sodium sulphate. The dried samples were then transferred into well-labelled and sterilized GC glass vials awaiting analysis.

3.4.2 GC-MS analysis

The phytochemical analysis was carried out using GC-MS (Agilent Technologies 7890A) equipment fitted with Agilent 7693 auto-sampler, a mass spectral library (NIST) version 08 and 11, with an acquisition software (Chemstation version B.02.02) in a workstation PC (HPZ 220 SFF) with an Intel Xeon processor. The instrument was fitted with an HP5MSUI column, Helium gas (99.99% purity) as a carrier gas, and Nitrogen (99.99% purity) gas as a collision gas. The electron impact mass spectra acceleration energy used was 70 eV, mass scanning speed was 40-550

m/z, and the filament delay time was 3.3 min. The flow rate of helium was set at one milliliter per minute, a splitless sample volume injection of 1 µL, with an ion source 230 ℃ at the ion source and 180 ºC quadrupole temperature. Filament delay time of 3.3 minutes and external quantification were used. The compounds' identities were determined according to their elution time and mass spectra, which were then compared with those of NIST 08 and 11 databases. Relative abundances of phytochemicals were determined using the relative peak areas of the total peak areas and tabulated.

3.5 Data management and statistical analysis

This study collected quantitative data from both the anthropometric and lipid profile assay, which was then tabulated and then organized using Microsoft Excel spreadsheets and transferred to Minitab v17 to be analysed. The data was analysed descriptively and then expressed as $\bar{x} \pm SEM$. Therafater, inferential statistics, One-factor Analysis of Variance (ANOVA) was computed to determine significant variations treatment groups. For pairwise comparison in the event of significant differences, Tukey's post hoc test was computed. During analysis, $p \leq 0.01$ was inferred to be significantly different. The results were presented using graphs and tables.

CHAPTER FOUR

RESULTS

4.1 Effects of *Piliostigma thonningii* and *Lonchocarpus eriocalyx* DCM extracts on anthropometric parameters of mice following DMPA-induced obesity

4.1.1 Effects of *Piliostigma thonningii* and *Lonchocarpus eriocalyx* DCM extracts on body weights of mice following DMPA-induced obesity

Generally, *P. thonningii* extract changed body weights of mice following DMPA-induced obesity (Table 4.1). Findings showed that body weights in normal control mice and obese control mice (negative control) significantly increased during the experiment ($p<0.01$). Nonetheless, the Orlistat-treated and extract-treated mice exhibited significantly lower ($p>0.01$) body weights in the entire experiment (Table 4.1).

During the 1st week of the study, the negative control mice showed substantially higher body weights than those noted ($p<0.01$) in the other treatment groups. Besides, the obese mice treated with *P. thonningii* extract at 75 and 150 mg/Kgbw, as well as those administered with Orlistat demonstrated no significant variations in body weights and were comparable with normal control mice ($p>0.01$). Notably, therapy with *P. thonningii* extract at 300 mg/Kgbw had significantly lower body weights relative to body weights ($p<0.01$) in the other treatment groups (Table 4.1).

During the 2nd week, the body weights of mice administered with Orisat and *P. thonningii* extract at 150 and 300 mg/Kgbw were statistically similar ($p>0.01$). Nevertheless, the obese control mice had considerably higher body weights relative to body weights seen ($p<0.01$) in the other experimental groups. In addition, the body weights of normal control mice were considerably higher than body weight in mice

treated ($p<0.01$) with the extract (at the three studied doses) and Orlistat (Table 4.1)

Findings also revealed no notable variations in body weights of mice treated with *P. thonningii* extract ($p>0.01$) at 75 and 150 mg/Kgbw in the 3rd week. Nevertheless, the effect of the extract at 300 mg/Kgbw significantly reduced body weights of mice compared to body weights seen in Orlistat-treated and extract-treated (dosage of 75 and 150 mg/Kgbw) ($p<0.01$) mice. In addition, the obese control mice showed considerable higher ($p<0.01$) body weights in contrast to those recorded in the other treatment groups (Table 4.1).

In the 4th week, the body weights of positive control mice and those treated with *P. thonningii* extract at 150 and 300 mg/Kgbw were statistically similar ($p>0.01$). Besides, the body weights noted extract-treated mice at the dosages of 75 and 150 mg/Kgbw were non-significant ($p>0.01$). Further, the body weights recorded in mice administered with Orlistat and the extract at the three doses were markedly lower ($p<0.01$) relative to body weights in normal control and negative control mice (Table 4.1).

Notably, *P. thonningii* extract reduced body weights dose-dependently at dose levels of 150 and 300 mg/kgbw (Table 4.1). The body weights of mice administered with *P. thonningii* extract at 150 and 300 mg/Kgbw statistically matched ($p>0.01$) those of mice treated with Orlistat throughout the treatment period (Table 4.1).

The body weights of mice in each treatment group were also compared in the entire treatment period. The normal control mice revealed significant weekly increase in

body weights ($p<0.01$). Nevertheless, the body weights of Orlistat-treated mice in the first and the second week of the study were non-significant ($p>0.01$). Besides, there was a notable reduction in body weights of positive control mice ($p<0.01$) in the third and fourth weeks of the experiment (Table 4.1).

Therapy with *P. thonningii* extract at 75 mg/Kgbw showed no significant variations in body weights ($p>0.01$) in the first and third weeks of the experiment. Similarly, at the same dosage, the body weights of mice were comparable ($p>0.01$) in the second and third weeks. However, in the fourth week, the body weights were notably reduced ($p<0.01$) compared with those recorded during the 1st, 2nd and 3rd weeks (Table 4.1).

Treatment of mice with the extract at 150 mg/Kgbw showed no significant variation in body weights ($p>0.01$) in the first and second weeks of the experiment. Similarly, at the same dosage, the body weights in the 2nd and the 3rd weeks were statistically similar ($p>0.01$). Besides, the body weights in the third and fourth weeks were statistically insignificant ($p>0.01$). However, the body weights in the fourth week were considerably lower ($p<0.01$) than those reported in the first and second weeks of the experiment. Further, the body weights of mice in the third week were considerably lower ($p<0.01$) than those noted in the first week (Table 4.1).

At 300 mg/Kgbw, the effect of *P. thonningii* extract did not significantly change ($p>0.01$) body weights in the 1st and 2nd weeks of the experiments (Table 4.1). Moreover, the body weights of mice administered with *P. thonningii* extract dosage of 300mg/Kgbw significantly reduced ($p<0.01$) from the third to the fourth week of the experiment (Table 4.1).

In this study, the weekly total body weights of mice in each treatment group were also analyzed. The negative control mice had considerably higher ($p<0.01$) body weights relative to body weights in extract-treated, normal control and positive control mice. Besides, the total body weights in mice administered with *P. thonningii* extract doses 150 and 300mg/Kgbw statistically matched those of mice ($p>0.01$) treated with Orlistat. Further, the total body weights of normal control mice were substantially higher than body weight ($p<0.01$) in extract-treated and positive control mice (Table 4.1).

Table 4.1: Effect of *Piliostigma thonningii* DCM extract on body weights of mice following DMPA-induced obesity

Treatment group	Percentage change in body weights				
	1st week	**2nd week**	**3rd week**	**4th week**	**Total change**
Normal Control	2.30 ± 0.12^{bD}	5.71 ± 0.25^{bC}	8.12 ± 0.55^{bB}	11.51 ± 0.27^{bA}	6.91 ± 0.19^{b}
Negative Control	8.74 ± 0.47^{aD}	18.29 ± 0.49^{aC}	24.62 ± 0.53^{aB}	32.86 ± 0.54^{aA}	21.13 ± 0.41^{a}
Positive control	$1.48\pm0.16b^{bcA}$	0.89 ± 0.10^{dA}	-2.67 ± 0.12^{dB}	-4.05 ± 0.10^{dC}	-1.09 ± 0.07^{d}
Extract (75 mg/Kgbw)	2.00 ± 0.35^{bAB}	3.31 ± 0.53^{cA}	1.00 ± 0.31^{cB}	-2.00 ± 0.34^{cC}	1.08 ± 0.22^{c}
Extract (150 mg/Kgbw)	1.09 ± 0.11^{bcA}	0.71 ± 0.35^{dAB}	-1.48 ± 0.71^{cdBC}	-3.55 ± 0.41^{cdC}	-0.81 ± 0.33^{d}
Extract (300 mg/Kgbw)	0.59 ± 0.10^{cA}	0.10 ± 0.10^{dA}	-2.38 ± 0.47^{dB}	-4.80 ± 0.12^{dC}	-1.62 ± 0.10^{d}

Results are presented as Mean ±SEM for 5 animals. Values that share the same superscript lowercase letter column-wise and those with same uppercase letters row-wise are statistically similar ($p>0.01$; one-factor ANOVA and Tukey's post hoc).

Moreover, *L. eriocalyx* extract reduced body weights of mice dose-depedently following DMPA-induced obesity (Table 4.2). In the 1st week, there was no significant variation noted in the body weights of mice that received *L. eriocalyx* extract at 300 mg/Kgbw, as well as Orlistat and were comparable to body weights noted ($p>0.01$) in normal control mice. However, the negative control mice recorded a notable increase ($p<0.01$) in body weights than those seen in the other treatment groups (Table 4.2).

During the second week, the body weights in the negative control mice were notably higher than the body weights observed ($p<0.01$) in mice of the other experimental groups (Table 4.2). The body weights of extract-treated mice at 75 mg/Kgbw and normal control mice were statistically insignificant ($p>0.01$). Similarly, the body weights noticed in mice administered with Orlistat and *L. eriocalyx* extract at 150 mg/Kgbw were statistically non-significant ($p>0.01$; Table 4.2).

In the 3rd week, the body weights of mice treated with *L. eriocalyx* extract dosages of 75 and 150 mg/Kgbw were statistically non-significant ($p<0.01$). Nevertheless, the body weights of negative control mice were substantially greater relative to the body weights ($p<0.01$) of mice in the other treatment groups (Table 4.2).

In the 4th week, the body weights of extract-treated mice at 150 and 300 mg/Kgbw and Orlistat were statistically insignificant ($p>0.01$). Nevertheless, these body weights were considerably reduced ($p<0.01$) relative to those noted in the other treatment groups. Besides, the negative control mice demonstrated considerably higher ($p<0.01$) body weights relative to those seen in the other treatment groups

(Table 4.2).

A comparison of the body weights of experimental mice across the study period was also conducted. Results indicated that body weights in negative and normal control mice were substantially higher throughout the experiment ($p<0.01$). Besides, no significant variations were seen in the body weights of positive control mice ($p>0.01$) in the first and second weeks of the experiment. Nonetheless, the body weights of Orlistat-treated mice were substantially higher ($p<0.01$) relative to those seen in the preceding weeks (Table 4.2).

The body weights of extract-treated mice dosage of 75 mg/Kgbw were non-significant in the first and second weeks, as well as in the third and fourth weeks ($p>0.01$). More so, the body weights recorded in mice administered with 150mg/Kgbw of the extract statistically matched in the second and third weeks ($p>0.01$). Nevertheless, the body weights observed in extract-treated mice at 150 mg/Kgbw were substantially higher ($p<0.01$) relative to those noted in each of the preceding weeks (Table 4.2).

The body weights of extrat-treated mice at dosage of 300 mg/Kgbw were statistically non-significant in the second and third weeks, as well as in the third and the fourth weeks ($p>0.01$). Nonetheless, the body weights extract-treated mice at the dosage of 300 mg/Kgbw were significantly lower in the 2^{nd}, 3^{rd}, and 4^{th} weeks than ($p<0.01$) in the first week (Table 4.2).

The total body weight was also determined across the study period. A substantial

increase ($p<0.01$) in total body weights was seen in obese control mice in contrast to the other treatment groups. Besides, the extract-treated mice at the dosage of 150 mg/Kgbw, as well as Orlistat-treated mice revealed no significant variations in ($p<0.01$) total body weights. Nevertheless, the extract dose level of 300 mg/Kgbw exhibited a notably higher ($p<0.01$) reduction in the total body weights than the extract at 75 and 150 mg/Kgbw (Table 4.2). The normal control mice had significantly higher total change in body weights ($p<0.01$) relative to those noted in extract-treated and Orlistat-treated mice (Table 4.2).

Table 4.2: Effect of *Lonchocarpus eriocalyx* DCM extract on body weights of mice following DMPA-induced obesity

Treatment group	Weekly percentage changes in body weights				
	1st Week	2nd Week	3rd Week	4th week	Total change
Normal Control	2.30 ± 0.12^{cdD}	5.71 ± 0.25^{bC}	8.12 ± 0.55^{Bb}	11.51 ± 0.27^{bA}	6.91 ± 0.19^{b}
Negative Control	8.74 ± 0.47^{aD}	18.29 ± 0.49^{aC}	24.62 ± 0.53^{Ab}	32.86 ± 0.54^{aA}	21.13 ± 0.41^{a}
Positive Control	1.48 ± 0.16^{bdA}	0.89 ± 0.10^{cA}	-2.67 ± 0.12^{deB}	-4.05 ± 0.10^{dC}	-1.09 ± 0.07^{d}
Extract (75 mg/Kgbw)	6.21 ± 0.09^{bA}	5.54 ± 0.55^{bA}	0.89 ± 0.10^{Cb}	-1.57 ± 0.77^{cB}	2.77 ± 0.20^{c}
Extract (150 mg/Kgbw)	4.22 ± 0.70^{cA}	0.31 ± 0.20^{cB}	-0.97 ± 0.48^{cdB}	-4.03 ± 0.09^{dC}	-0.12 ± 0.19^{d}
Extract (300 mg/Kgbw)	1.19 ± 0.12^{dA}	-2.28 ± 0.58^{dB}	-3.89 ± 0.14^{eBC}	-4.98 ± 0.20^{dC}	-2.49 ± 0.16^{e}

Results are presented as Mean ±SEM for 5 animals. Values that share the same superscript lowercase letter column-wise and those with same uppercase letters row-wise are statistically similar ($p>0.01$; one-factor ANOVA and Tukey's post hoc).

In comparison, the obese mice that received *P. thonningii* extract showed significantly lower body weights than body weights of mice treated with *L. eriocalyx* extract at all doses ($p<0.01$) in week one of the experiment (Figure 4.1). Nonetheless, in the second and third weeks, the body weights of mice that received the two studied extracts at 75 and 150 mg/Kgbw were statistically similar ($p>0.01$). Besides, the mice treated with *L. eriocalyx* extract dosage of 300 mg/Kgbw had significantly lower body weights than those in mice treated with *P. thonningii* extract at the corresponding dosage ($p<0.01$). Further, in the 4^{th} week of the experiment, the obese mice treated with the two extracts at the same dosage statistically matched ($p>0.01$) in body weights (Figure 4.1).

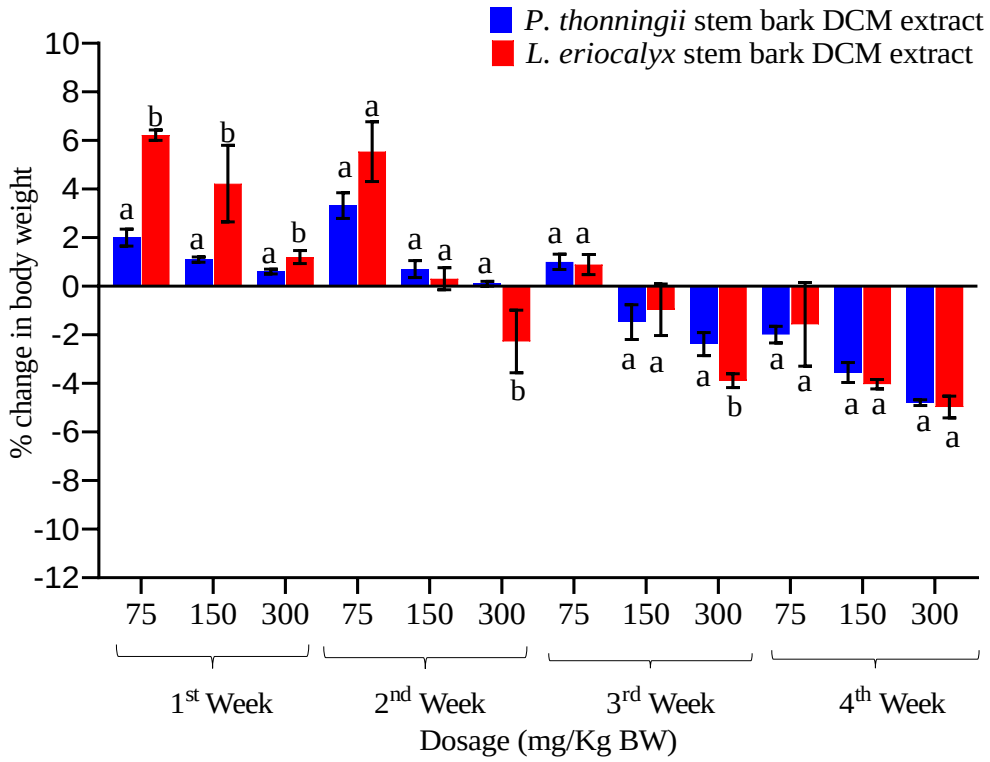


Figure 4.1: Comparison of effects of *Piliostigma thonningii* and *Lonchocarpus eriocalyx* DCM extracts on body weights following DMPA-induced obesity in mice

Bars that share the same letter at the same dosage and week are statistically similar using independent t-test ($p>0.01$).

4.1.2 Effects of *Piliostigma thonningii* and *Lonchocarpus eriocalyx* DCM extracts on relative organ weights of mice following DMPA-induced obesity

This study determined relative organ weights of brain, spleen, kidneys, lungs and liver in mice that received DCM extract of *P. thonningii* following DMPA-induced obesity. Therapy with *P. thonningii* extract caused alterations in relative organ weights in obese mice (Table 4.3).

The relative weights of the spleen, brain, lungs, and kidneys in mice administered with *P. thonningii* extract at the three dosages were statistically non-significant ($p>0.01$) and were comparable to the relative weights observed in Orlistat-treated mice. Nonetheless, the relative weights of liver in mice treated with the extract at the three dosages differed significantly ($p<0.01$). In addition, the relative weights of

lungs, spleen, liver, heart, kidneys, and brain in obese control mice were substantially higher compared to the relative weights reported ($p<0.01$) in the other treatment groups. Further, the normal control mice had significantly lower relative weights of lungs, spleen, heart, kidneys, and brain compared to relative weights reported ($p<0.01$) in the other treatment groups (Table 4.3).

Table 4.3: Effect of *Piliostigma thonningii* DCM extract on relative organ weights of mice following DMPA-induced obesity

Treatment group	Relative organ weight (%)					
	Brain	**Liver**	**Heart**	**Lungs**	**Spleen**	**Kidneys**
Normal Control	1.46±0.01^{c}	5.93±0.02^{c}	0.48±0.02^{b}	0.56±0.02^{c}	1.28±0.02^{c}	1.00±0.02^{c}
Negative Control	1.70±0.01^{a}	8.10±0.11^{a}	0.77±0.02^{a}	0.84±0.01^{a}	1.52±0.01^{b}	1.32±0.02^{a}
Positive control	1.58±0.01^{b}	6.20±0.08^{c}	0.56±0.02^{b}	0.65±0.01^{b}	1.39±0.02^{a}	1.12±0.02^{b}
Extract (75 mg/Kgbw)	1.57±0.01^{b}	6.72±0.13^{b}	0.56±0.02^{b}	0.68±0.01^{b}	1.40±0.02^{a}	1.15±0.02^{b}
Extract (150 mg/Kgbw)	1.54±0.01^{b}	6.29±0.04^{bc}	0.55±0.02^{b}	0.65±0.01^{b}	1.40±0.02^{a}	1.14±0.02^{b}
Extract (300 mg/Kgbw)	1.54±0.01^{b}	6.17±0.04^{c}	0.55±0.01^{b}	0.63±0.02^{b}	1.39±0.01^{a}	1.10±0.02^{b}

Results are presented as Mean ±SEM for 5 animals. Values that share the same superscript lowercase letter column-wise are statistically similar ($p>0.01$; one-factor ANOVA and Tukey's post hoc).

Moreover, treatment of obese mice with *L. eriocalyx* DCM extract resulted in alterations in the relative organ weights of mice following DMPA induced obesity (Table 4.4). The relative weights of the spleen, brain, liver, heart, kidneys, and lungs recorded in obese control mice were considerably higher than those noted ($p>0.01$) in Orlistat-treated, extract-treated and healthy control mice (Table 4.4).

The effect of *L. eriocalyx* on the relative weights of the spleen, liver, heart, brain, kidneys and lungs was non-significant at the three doses ($p>0.01$). Similarly, the effect of Orlistat on the relative weights of the spleen, brain, heart, kidneys and lungs statistically matched the effect of the extract ($p>0.01$) at the three studied dosages. However, the relative organ weights of normal control mice were significantly greater than the relative weights reported ($p<0.01$) in the other treatment groups. Further, the relative organ weights of the spleen, brain, heart, kidneys and lungs were considerably lower than the relative weights ($p<0.01$) in the other treatment groups (Table 4.4).

Table 4.4: Effect of *Lonchocarpus eriocalyx* DCM extract on relative organ weights of mice following DMPA-induced obesity

Treatment group	Relative organ weight (%)					
	Brain	**Lungs**	**Liver**	**Spleen**	**Heart**	**Kidneys**
Normal Control	1.46±0.01^{c}	0.56±0.02^{c}	5.93±0.02^{d}	1.28±0.02^{c}	0.48±0.02^{b}	1.00±0.02^{c}
Negative Control	1.70±0.01^{a}	0.84±0.01^{a}	8.10±0.11^{a}	1.52±0.01^{a}	0.77±0.02^{a}	1.32±0.01^{a}
Positive Control	1.58±0.01^{b}	0.65±0.01^{b}	6.20±0.08^{cd}	1.39±0.02^{b}	0.56±0.02^{b}	1.32±0.01^{b}
Extract (75 mg/Kgbw)	1.58±0.02^{b}	0.72±0.01^{b}	6.74±0.08^{b}	1.43±0.01^{b}	0.56±0.02^{b}	1.17±0.03^{b}
Extract (150 mg/Kgbw)	1.57±0.02^{b}	0.72±0.02^{b}	6.62±0.04^{b}	1.41±0.02^{b}	0.55±0.01^{b}	1.15±0.01^{b}
Extract (300 mg/Kgbw)	1.57±0.01^{b}	0.70±0.02^{b}	6.46±0.06^{bc}	1.39±0.02^{b}	0.56±0.02^{b}	1.13±0.02^{b}

Results are presented as Mean ±SEM for 5 animals. Values that share the same superscript lowercase letter column-wise are statistically similar ($p>0.01$; one-factor ANOVA and Tukey's post hoc).

Comparatively, the relative liver weights in mice treated with *P. thonningii* extract were significantly lower than relative weights recorded in mice administered with *L. eriocalyx* extract at the corresponding dosages ($p<0.01$) of 150 and 300 mg/Kgbw. However, the relative weights of liver in mice treated with the two extracts at the same dosage of 75 mg/Kgbw matched statistically ($p>0.01$). Besides, the effects of the two extracts at the same dosages statistically matched the relative weights of the spleen, brain, heart, kidneys and lungs ($p>0.01$; Figure 4.2).

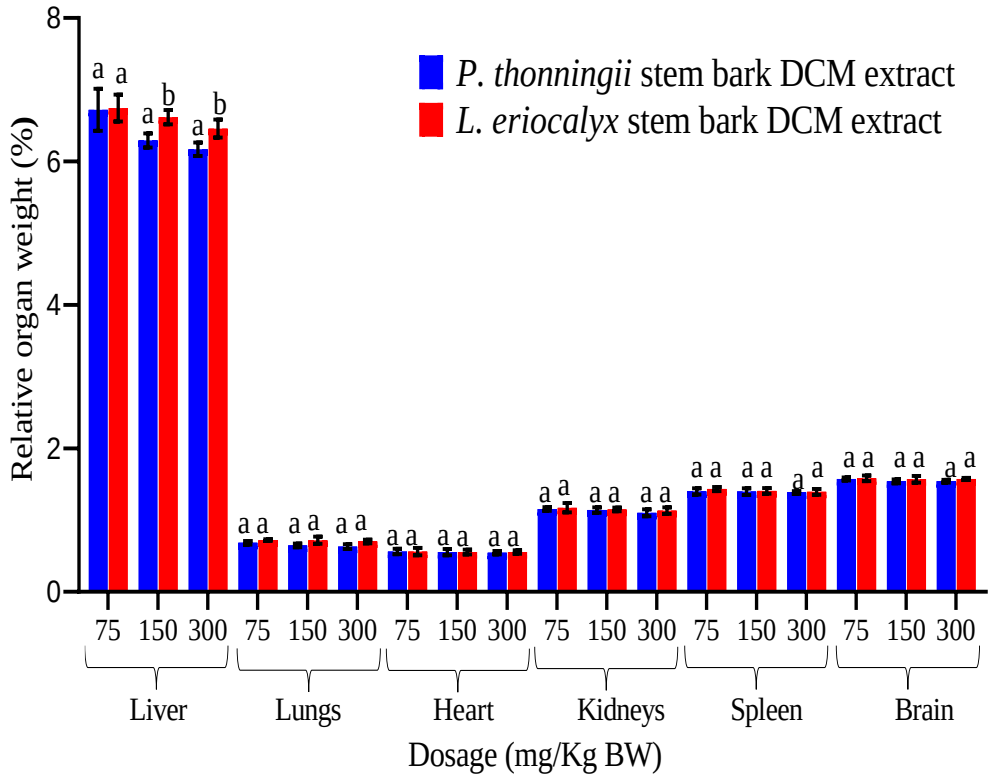


Figure 4.2: Comparison of effects of *Piliostigma thonningii* and *Lonchocarpus eriocalyx* DCM extracts on relative organ weights following DMPA-induced obesity in mice.
Bars that share the same letter at the same dosage and week are statistically similar using independent t-test ($p>0.01$).

4.1.3 Effects of *Piliostigma thonningii* and *Lonchocarpus eriocalyx* DCM extracts on fat pad weights of mice following DMPA-induced obesity

The fat pad weights of DMPA-induced obese mice administered with *P. thonningii* DCM extract were also determined. The results revealed no considerable variations ($p>0.01$) in fat regions of Orlistat-treated and extract-treated mice. Conversely, negative control mice had significantly higher fat pad weights ($p<0.01$) in each of the determined fat regions than the other experimental groups (Table 4.5).

The brown adipose tissue (BAT), subcutaneous (SC), parametrial (PM), retroperitoneal (RP), mesenteric (MS), pararenal (PR) and total fat content (TFC) weights of obese mice treated with *P. thonningii* extract at the three dosages were insignificant and were comparable to weights noted in Orlistat-treated mice ($p>0.01$). Nevertheless, the weights of BAT, SC, PM, RP, MS, PR and TFC in obese control mice were considerably higher than the weights noted ($p>0.01$) in the other treatment groups. Notably, the weights of BAT, SC, PM, RP, MS, and PR normal control mice were non-significant compared with those reported ($p>0.01$) in extract-treated mice at the three dosages (Table 4.5).

Table 4.5: Effect of *Piliostigma thonningii* DCM extract on Fat pad weights of mice following DMPA-induced obesity

Treatment group	Fat pad weight (g)						
	PR	**SC**	**MS**	**RP**	**PM**	**BAT**	**TFC**
Normal Control	0.04±0.01^{b}	0.15±0.01^{b}	0.04±0.01^{b}	0.03±0.01^{b}	0.07±0.01^{b}	0.03±0.01^{b}	0.36±0.01^{b}
Negative Control	0.14±0.01^{a}	1.09±0.03^{a}	0.20±0.01^{a}	0.15±0.01^{a}	0.19±0.01^{a}	0.08±0.01^{a}	1.86±0.03^{a}
Positive control	0.03±0.01^{b}	0.13±0.01^{b}	0.03±0.01^{b}	0.02±0.01^{b}	0.06±0.01^{b}	0.03±0.01^{b}	0.29±0.01^{bc}
Extract (75 mg/Kgbw)	0.03±0.01^{b}	0.12±0.01^{b}	0.03±0.01^{b}	0.03±0.01^{b}	0.07±0.01^{b}	0.03±0.01^{b}	0.31±0.01^{bc}
Extract (150 mg/Kgbw)	0.03±0.01^{b}	0.10±0.01^{b}	0.03±0.01^{b}	0.03±0.01^{b}	0.05±0.01^{b}	0.03±0.01^{b}	0.27±0.01^{c}
Extract (300 mg/Kgbw)	0.03±0.01^{b}	0.09±0.01^{b}	0.03±0.01^{b}	0.03±0.01^{b}	0.05±0.01^{b}	0.03±0.01^{b}	0.25±0.01^{c}

Results are shown as Mean ±SEM for 5 animals. Values that share the same superscript lowercase letter column-wise are statistically similar ($p>0.01$; one-factor ANOVA and Tukey's post hoc).

BAT = Brown Adipose Tissue; SC = Subcutaneous; PM = Parametrial; RP = Retroperitoneal; MS = Mesenteric; PR = Pararenal; TFC = Total Fat Content

On the other hand, the weights of fat pads of positive control mice, and those that received *L. eriocalyx* extracts at the three dosess were statistically similar ($p>0.01$). However, the obese control mice had considerably higher fat pad weights in each of the determined regions than those noticed in mice of the other treatment groups (Table 4.6).

The weights of BAT, SC, PM, RP, MS, PR and TFC in extract-treated mice at the three dosages were insignificant and were comparable to weights observed in Orlistat-treated mice at the three studied dosages ($p>0.01$). Nonetheless, the weights of BAT, SC, PM, RP, MS, PR and TFC were substantially higher than the weights reported ($p<0.01$) in mice of the other treatment groups. Besides, in the normal control mice, the weights of BAT, SC, PM, RP, MS and PR statistically matched those seen in extract-treated mice ($p>0.01$) at the three doses (Table 4.6).

Table 4.6: Effect of *Lonchocarpus eriocalyx* DCM extract on Fat pad weights of mice following DMPA-induced obesity

Treatment group	Fat pad weight (g)						
	SC	**BAT**	**TFC**	**PR**	**RP**	**PM**	**MS**
Normal Control	0.15±0.01^{b}	0.03±0.01^{b}	0.36±0.01^{b}	0.04±0.01^{b}	0.03±0.01^{b}	0.07±0.01^{b}	0.04±0.01^{b}
Negative Control	1.09±0.03^{a}	0.08±0.01^{a}	1.86±0.03^{a}	0.14±0.01^{a}	0.15±0.01^{a}	0.19±0.01^{a}	0.20±0.01^{a}
Positive Control	0.13±0.01^{b}	0.03±0.01^{b}	0.29±0.01^{bc}	0.03±0.01^{b}	0.02±0.01^{b}	0.06±0.01^{b}	0.03±0.01^{b}
Extract (75 mg/Kgbw)	0.13±0.01^{b}	0.03±0.01^{b}	0.34±0.01^{bc}	0.04±0.01^{b}	0.04±0.01^{b}	0.06±0.01^{b}	0.04±0.01^{b}
Extract (150 mg/Kgbw)	0.12±0.01^{b}	0.03±0.01^{b}	0.30±0.01^{bc}	0.03±0.01^{b}	0.03±0.01^{b}	0.05±0.01^{b}	0.03±0.01^{b}
Extract (300 mg/Kgbw)	0.12±0.01^{b}	0.02±0.01^{b}	0.26±0.01^{c}	0.02±0.01^{b}	0.02±0.01^{b}	0.04±0.01^{b}	0.03±0.01^{b}

Results are shown as Mean ±SEM for 5 animals. Values that share the same superscript lowercase letter column-wise are statistically similar ($p>0.01$; one-factor ANOVA and Tukey's post hoc).

BAT = Brown Adipose Tissue; SC = Subcutaneous; PM = Parametrial; RP = Retroperitoneal; MS = Mesenteric; PR = Pararenal; TFC = Total Fat Content.

In comparison, the effects *P. thonningii* and *L. eriocalyx* DCM extracts on weights of BAT, PM, RP, MS and PR were statistically similar at the three studied dosages ($p>0.01$). Similarly, the SC weights in mice that received the two extracts dosage of 75 mg/Kgbw were non-significant ($p>0.01$). However, The SC of mice treated with extract of *P. thonningii* at the dosages of 150 and 300 mg/Kgbw were substantially lower relative to SC seen in mice that received *L. eriocalyx* ($p<0.01$) at the equivalent dosage (Figure 4.3).

Additionally, the total fat contents obtained in mice treated with 75 and 300 mg/Kgbw doses of the two plant extracts were statistically non-significant ($p>0.01$). Nonetheless, the total fat contents measured in mice that received 150 mg/Kgbw of *P. thonningii* extract were considerably lower relative to those seen in mice administered the same dose of *L. eriocalyx* extract ($p<0.01$; Figure 4.3).

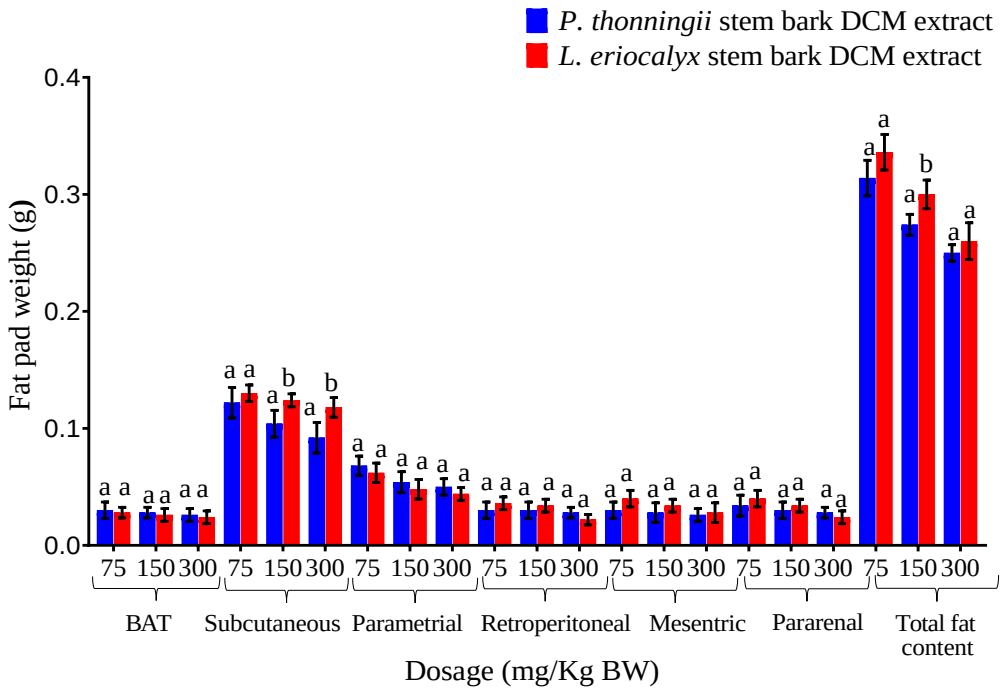


Figure 4.3: Comparison of effects of *Piliostigma thonningii* and *Lonchocarpus eriocalyx* DCM extracts on fat pad weights following DMPA-induced obesity in mice.
Bars that share the same letter at the same dosage are statistically similar using independent t-test ($p>0.01$).

4.1.4 Effects of *Piliostigma thonningii* and *Lonchocarpus eriocalyx* DCM extracts on Body Mass Indices of mice following DMPA-induced obesity

The study findings established that therapy with *P. thonningii* DCM extract affected the body mass index (BMI) following DMPA-induced obesity in mice. The BMI of normal control and positive control mice, as well as those treated with extract of *P. thonningii* at the three dosages in the first week, were statistically alike ($p>0.01$). Conversely, in the same week, the negative control mice recorded considerably higher ($p<0.01$) BMI relative to the BMI of mice reported in the other treatment groups (Table 4.7).

In the second week, the BMI reported in normal control, Orlistat-treated and extract-treated (at the dosages of 75 and 150 mg/Kgbw) mice ($p>0.01$) were statistically insignificant. However, in the same period, negative control mice showed considerably higher ($p<0.01$) BMI relative to BMI seen in the other treatment groups (Table 4.7). Notably, the BMI of mice that received 300mg/Kgbw of *P. thonningii* extract were considerably lower than the BMI ($p<0.01$) of mice in the other treatment groups (Table 4.7).

In the 3rd week, the BMI of mice treated with *P. thonningii* extract at all studied dosages were statistically similar and matched BMI reported in Orlistat-treated mice ($p>0.01$). Besides, the BMI of negative control mice were notably higher ($p<0.01$) relative to BMI seen in the other treatment groups. The BMI of the normal control mice were statistically greater than the BMI ($p<0.01$) of mice that received Orlistat and the extract at the three dosages (Table 4.7).

In the fourth week, the BMI of extract-treated mice at the three dosages were

statistically non-significant and matched ($p>0.01$) the BMI of Orlistat-treated mice. Nonetheless, the negative control mice exhibited considerably higher BMI than BMI reported ($p<0.01$) in the other experimental groups. In addition, the normal control mice had considerably higher BMI relative to those recorded ($p<0.01$) in Orlistat-treated and extract-treated mice (Table 4.7).

The total change in BMI of extract-treated (at the three dosages) mice never differed significantly and were comparable to BMI reported ($p>0.01$) in Orlistat-treated mice. Nonetheless, the obese control mice had significantly higher total change in BMI compared to total change noted ($p<0.01$) in the other treatment groups. Besides, the normal control mice had significantly higher total change in BMI than total change ($p<0.01$) in mice treated with the extract (at the three dosages) and Orlistat (Table 4.7).

This study also analyzed the BMI of experimental mice across the treatment period (Table 4.7). The BMI of normal control mice increased significantly in weeks 3 and 4 relative to ($p<0.01$) weeks 1 and 2 of the experiment. Similarly, the obese control mice had significantly higher BMI in the fourth week in contrast to weeks 1, 2 and 3 ($p<0.01$). Nevertheless, the Orlistat-treated mice showed substantial reduction in BMI in week 4 compared to ($p<0.01$) weeks 1, 2 and 3 of the experiment. Notably, the effect of *P. thonningii* extract at the dosage of 75 mg/Kgbw noted no substantial change in BMI ($p>0.01$) throughout the experiment. In addition, the BMI extract-treated mice at the dose of 150 mg/kg bw were considerably reduced in week four relative to weeks 1 and 2 ($p<0.01$). Further, the BMI of mice that received extract at the dosage of 300 mg/Kgbw were significantly reduced in week four compared in

weeks 1, 2 and 3 ($p<0.01$) of the experiment (Table 4.7).

Table 4.7: Effect of *Piliostigma thonningii* DCM extract on Body Mass Indices of mice following DMPA-induced obesity

Treatment group	Percentage change in BMI				
	1st Week	2nd Week	3rd Week	4th week	Total change
Normal Control	0.76 ± 0.04^{bB}	0.75 ± 0.08^{bB}	1.29 ± 0.08^{bA}	1.46 ± 0.08^{bA}	1.07 ± 0.04^{b}
Negative Control	1.71 ± 0.27^{aC}	3.92 ± 0.18^{aB}	4.83 ± 0.30^{aB}	6.18 ± 0.12^{aA}	4.16 ± 0.19^{a}
Positive control	0.49 ± 0.05^{bA}	0.30 ± 0.03^{bcAB}	0.20 ± 0.04^{cB}	-0.27 ± 0.03^{cC}	0.18 ± 0.02^{c}
Extract (75 mg/Kgbw)	0.66 ± 0.12^{bA}	0.65 ± 0.18^{bcA}	0.33 ± 0.10^{cA}	-0.01 ± 0.12^{cA}	0.41 ± 0.14^{c}
Extract (150 mg/Kgbw)	0.36 ± 0.04^{bA}	0.23 ± 0.11^{bcA}	-0.06 ± 0.05^{cAB}	-0.32 ± 0.11^{cB}	0.05 ± 0.03^{c}
Extract (300 mg/Kgbw)	0.20 ± 0.03^{bA}	0.03 ± 0.03^{cA}	-0.14 ± 0.12^{cA}	-0.53 ± 0.04^{cB}	-0.11 ± 0.04^{c}

Results are presented as Mean ±SEM for 5 mice. Values that share the same superscript lowercase letter column-wise and those with same uppercase letters row-wise are statistically similar ($p>0.01$; one-factor ANOVA and Tukey's post hoc).

The effect of *L. eriocalyx* extract at the three dosages (75, 150 and 300 mg/Kgbw) caused alterations in BMI following DMPA-induced obesity in mice (Table 4.8). In the entire treatment period, the obese control mice reported the highest BMI than BMI in the other treatment groups ($p<0.01$). Therapy with *L. eriocalyx* extract and Orlistat lowered the BMI of obese mice throughout the treatment period (Table 4.8).

In the first week, the effect of *L. eriocalyx* extract on BMI was non-significant at the three dosages and statistically matched the effect of Orlistat ($p>0.01$). However, the obese control mice had significantly higher BMI relative to BMI noted in mice administered the extract (at the three doses) and Orlistat ($p<0.01$). In addition, the BMI of mice in normal control mice were considerably higher than BMI seen ($p<0.01$) in Orlistat-treated and extract-treated mice (Table 4.8).

In the 2nd week, mice treated with *L. eriocalyx* extract doses of 150, 300mg/Kgbw and positive control mice showed comparable ($p>0.01$) BMI. Similarly, the positive control and normal control mice showed statistically similar ($p>0.01$) BMI. In the same week, normal control group mice showed statistically similar ($p>0.01$) BMI with mice that received *L. eriocalyx* extract at 75 mg/Kgbw. However, the negative control mice had a significantly higher BMI than BMI ($p<0.01$) reported in mice of the other treatment groups (Table 4.8).

In the third week, the BMI in mice administered with *L. eriocalyx* extract at all tested doses as well as BMI in Orlistat control control were statistically insignificant ($p>0.01$). Conversely, the BMI of the negative control mice were considerably higher relative to the BMI seen ($p<0.01$) in mice of the other treatment groups. Besides, the

normal control mice had substantially higher BMI in contrast to BMI seen in mice administered with ($p<0.01$) the extract (at the three dosages) and Orlistat (Table 4.8).

In the fourth week, the obese mice treated with the extract at the three dosages differed significantly ($p<0.01$). Nevertheless, the BMI of extract-treated mice statistically matched BMI recorded in Orlistat-treated mice ($p>0.01$). Besides, the obese control mice had considerably higher BMI relative to BMI in mice of ($p<0.01$) the other treatment groups. Further, the BMI of the normal control mice were substantially higher than BMI noted ($p<0.01$) in Orlistat-treated and extract-treated mice (Table 4.8).

The total change in BMI of extract-treated mice were comparable whereas at dosage of 75 and 300mg/kgbw the effects were significantly different ($p<0.01$). Nevertheless, the effect of Orlistat on BMI statistically matched the effect of extract at the three studied dosages ($p>0.01$). In addition, the BMI of obese control mice were considerably higher relative to the BMI seen ($p<0.01$) in mice of the other treatment groups. Further, the BMI of normal control mice were considerably greater than BMI in mice treated with ($p<0.01$) the extract (at the three doses) and Orlistat (Table 4.8).

In analysis across the treatment period, the BMI of normal control mice increased significantly in weeks 3 and 4 relative to ($p<0.01$) weeks 1 and 2. Similarly, the negative control mice revealed significant increase in BMI in the fourth week compared to weeks 1, 2 and 3 of the experiment ($p<0.01$). However, the Orlistat-treated mice significantly reduced BMI in week 4 in contrast to weeks 1, 2 and 3 ($p<0.01$). Besides, the BMI recorded in mice that received *L. eriocalyx* extract at 75

and 300 mg/Kgbw were considerably lower in week four compared to ($p<0.01$) weeks 1, 2 and 3. Further, the BMI of mice that received *L. eriocalyx* extract dose level of 150 mg/Kgbw were substantially lower in week four compared to ($p<0.01$) weeks 1 and 2 of the experiment (Table 4.8).

Table 4.8: Effect of *Lonchocarpus eriocalyx* DCM extract on Body Mass Indices of mice following DMPA-induced obesity

Treatment group	Percentage change in BMI				
	week 1	**week 2**	**week 3**	**week 4**	**Total change**
Normal Control	0.76 ± 0.04^{bB}	0.75 ± 0.08^{bcB}	1.29 ± 0.08^{bA}	1.46 ± 0.08^{bA}	1.07 ± 0.04^{b}
Negative Control	1.71 ± 0.27^{aC}	3.92 ± 0.18^{aB}	4.83 ± 0.30^{aB}	6.18 ± 0.12^{aA}	4.16 ± 0.19^{a}
Positive Control	0.49 ± 0.05^{bA}	0.30 ± 0.03^{cdAB}	0.20 ± 0.04^{cB}	-0.27 ± 0.03^{cdC}	0.18 ± 0.02^{cd}
Extract (75 mg/Kgbw)	0.92 ± 0.03^{bA}	0.92 ± 0.05^{bA}	0.30 ± 0.06^{cB}	-0.09 ± 0.03^{cC}	0.51 ± 0.01^{c}
Extract (150 mg/Kgbw)	0.50 ± 0.06^{bA}	0.10 ± 0.07^{dB}	-0.10 ± 0.06^{cBC}	-0.27 ± 0.03^{cdC}	0.06 ± 0.02^{d}
Extract (300 mg/Kgbw)	0.40 ± 0.04^{bA}	0.11 ± 0.04^{dB}	-0.21 ± 0.04^{cC}	-0.59 ± 0.07^{dD}	-0.07 ± 0.07^{d}

Results are presented as Mean ±SEM for 5 mice. Values that share the same superscript lowercase letter column-wise and those with same uppercase letters row-wise are statistically similar ($p>0.01$; one-factor ANOVA and Tukey's post hoc).

In comparison, the was no substantial variation in the BMI of mice treated with the two extracts at the three studied dosages in the second, third and fourth weeks of the experiment ($p>0.01$). Similarly, in the first week of the experiment, the BMI of mice treated with the two extracts at 75 and 150 mg/Kgbw ($p>0.01$) were statistically insignificant. However, in the same period, the mice that received *P. thonningii* extract at 300 mg/Kgbw had significantly lower BMI ($p<0.01$) compared to BMI seen in mice administered *L. eriocalyx* extract at the equivalent dosage (Figure 4.4).

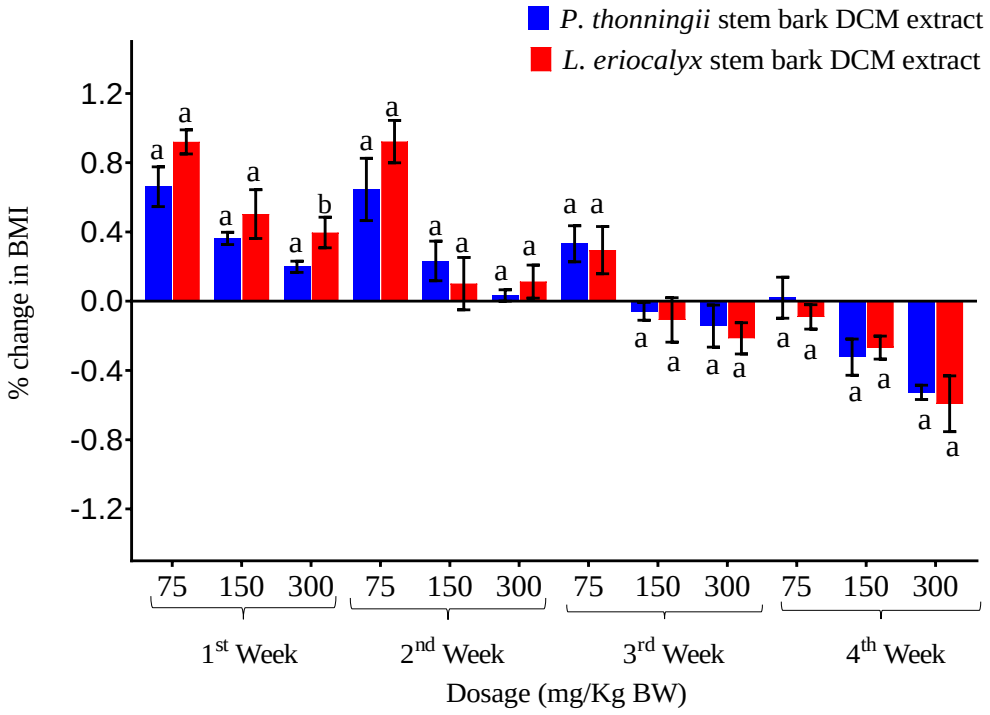


Figure 4.4: Comparison of effects of *Piliostigma thonningii* and *Lonchocarpus eriocalyx* DCM extracts on BMI following DMPA-induced obesity in mice.
Bars that share the same letter at the same dosage and week are statistically similar using independent t-test ($p>0.01$).

4.1.5 Effects of *Piliostigma thonningii* and *Lonchocarpus eriocalyx* DCM extracts on abdominal circumferences of mice following DMPA-induced obesity

As depicted in table 4.9, therapy with *P. thonningii* DCM extract caused changes in abdominal circumferences of mice following DMPA-induced obesity. The obese control mice had the highest abdominal circumferences ($p<0.01$) throughout the

experiment. The effect of *P. thonningii* extract at the three studied dosages as well as Orlistat ameliorated increased abdominal circumferences than those reported in obese control mice ($p<0.01$).

In the 1st, 2nd, 3rd and and 4th weeks of the experiment, the effect *of P. thonningii* extract at the three studied dosages on abdominal circumferences did not differ significantly ($p<0.01$) in obese mice. The effect of the extract reduced abdominal circumference dose-dependently in obese mice (Table 4.9). The abdominal circumferences of Orlista-treated mice statistically matched those of extract-treated mice at the dosage of 300 mg/Kgbw in the second and third weeks of the study ($p>0.01$). Similarly, the effect of Orlistat statistically matched the effect of the extract dosages of 150 and 300 mg/Kgbw in the first week ($p>0.01$) of the experiment. In addition, the obese control mice had substantially higher abdominal circumferences than those reported in normal control, Orlista-treated and extract-treated mice in the entire study ($p<0.01$). Further, the normal control mice had considerably higher abdominal circumference compared to Orlistat-treated and extract-treated mice in the third and fourth weeks ($p<0.01$) of the experiment (Table 4.9).

The total change in the abdominal circumferences in mice treated with the extract at the three studied dosages differed considerably in obese mice ($p<0.01$). Nevertheless, the Orlistat-treated mice had total change in abdominal circumferences that statistically matched those seen in extract-treated mice at the dose of 300 mg/Kgbw ($p>0.01$). Besides, the total change in abdominal circumferences of obese control mice were statistically higher than the total change noticed ($p<0.01$) in normal control, Orlistat-treated and extract-treated mice. Further, the normal control mice had

abdominal circumferences that were considerably greater than those ($p<0.01$) in Orlistat-treated and extract-treated mice (Table 4.9).

In analysis across the treatment period, the normal control mice had considerably higher abdominal circumference in the fourth week compared to weeks 1, 2 and 3 of the study ($p<0.01$). Similarly, the negative control mice revealed significant increase in abdominal circumference from week one to week four of the experiment ($p<0.01$). However, the effect of Orlistat revealed considerable reduction in abdominal circumferences ($p<0.01$) in the entire experiment. In addition, the effect of the extract at the three studied dosages had substantial reduction in abdominal circumferences in the fourth week than in the first, second and third weeks of the experiment ($p<0.01$; Table 4.9).

Table 4.9: Effect of *Piliostigma thonningii* DCM extract on abdominal circumference of mice following DMPA-induced obesity

Treatment group	Percentage changes in abdominal circumferences				
	1st Week	2nd Week	3rd Week	4th week	Total change
Normal Control	2.45 ± 0.02^{bC}	4.77 ± 0.04^{bB}	5.00 ± 0.24^{bB}	7.21 ± 0.24^{bA}	4.86 ± 0.13^{b}
Negative Control	6.44 ± 0.25^{aD}	10.40 ± 0.49^{aC}	12.36 ± 0.38^{aB}	16.01 ± 0.31^{aA}	11.30 ± 0.28^{a}
Positive control	2.47 ± 0.02^{cA}	0.49 ± 0.30^{dB}	-4.39 ± 0.33^{eC}	-8.00 ± 0.08^{eD}	-2.36 ± 0.12^{e}
Extract (75 mg/Kgbw)	3.46 ± 0.23^{bA}	3.71 ± 0.37^{bcA}	1.46 ± 0.24^{cB}	-2.02 ± 0.31^{cC}	1.65 ± 0.21^{c}
Extract (150 mg/Kgbw)	1.94 ± 0.29^{cA}	2.91 ± 0.30^{cA}	-1.98 ± 0.31^{dB}	-5.38 ± 0.28^{dC}	-0.63 ± 0.12^{d}
Extract (300 mg/Kgbw)	2.47 ± 0.03^{cA}	1.24 ± 0.02^{dA}	-4.92 ± 0.47^{eB}	-10.96 ± 0.28^{fC}	-3.04 ± 0.18^{e}

Results are presented as Mean ±SEM for 5 animals. Values that share the same superscript lowercase letter column-wise and those with same uppercase letters row-wise are statistically similar ($p>0.01$; one-factor ANOVA and Tukey's post hoc).

Moreover, the effect of *L. eriocalyx* extract in obese mice resulted in alterations of the abdominal circumferences following DPMA-induced obesity (Table 4.10). The negative control mice had significantly higher abdominal circumferences ($p<0.01$) throughout the experiment (Table 4.10). Treatment with *L. eriocalyx* DCM extract at the three dosages as well as Orlistat caused reduction in abdominal circumferences in the entire study (Table 4.10).

The effect of *L. eriocalyx* DCM extract at the three studied dosages demonstrated significant variations in abdominal circumferences of obese mice ($p<0.01$) in the entire experiment (Table 4.10). The effect of the extract ameliorated increased abdominal circumferences in a dose-dependent manner (Table 4.10). The effect of Orlistat on abdominal circumference statistically matched the effect of the extract dosage of 300 mg/Kgbw in the second and third weeks of the experiment ($p>0.01$). Similarly, in the first week of the experiment, the abdominal circumferences of Orlistat-treated and extract-treated at dosages of 150 and 300 mg/Kgbw were statistically non-significant ($p>0.01$). In addition, the obese control mice reported the highest abdominal circumferences compared to circumferences seen ($p<0.01$) in the other treatment groups. Further, the normal control mice had significantly higher abdominal circumferences than circumferences noted in Orlistat-treated and extract-treated mice in the third and fourth weeks ($p<0.01$) of the experiment (Table 4.10).

The total abdominal circumferences of extract-treated mice at the three dosages differed considerably ($p<0.01$) in obese mice. Nevertheless, the effect of Orlistat on the total change in abdominal circumferences statistically matched the effect of the extract ($p<0.01$) at the dosage of 300 mg/Kgbw. Besides, the obese control mice had

considerably higher total change in abdominal circumferences relative to total changes reported ($p<0.01$) in mice of the other treatment groups. Further, the normal control mice had substantially higher total changes in abdominal circumferences than those noted ($p<0.01$) in Orlistat-treated and extract-treated mice (Table 4.10).

The abdominal circumferences of mice in different treatment groups were also compared across the four-week study period. The abdominal circumferences of normal control mice were significantly higher in the fourth week compared to weeks 1, 2 and 3 of the experiment ($p<0.01$). Similarly, the obese control mice had significantly higher abdominal circumference ($p<0.01$) throughout the experiment. However, the Orlistat-treated and extract-treated mice had significantly lower abdominal circumferences in the fourth week compared to the first, second and third weeks ($p<0.01$) of the experiment (Table 4.10).

Table 4.10: Effect of *Lonchocarpus eriocalyx* DCM extract on abdominal circumferences of mice following DMPA-induced obesity

Treatment group	Percentage changes in abdominal circumferences				
	1st Week	2nd Week	3rd Week	4th week	Total change
Normal Control	2.45±0.02^{bC}	4.77±0.04^{bB}	5.00±0.24^{bB}	7.21±0.24^{bA}	4.86±0.13^{b}
Negative Control	6.44±0.25^{aD}	10.40±0.49^{aC}	12.36±0.38^{aB}	16.01±0.31^{aA}	11.30±0.28^{a}
Positive Control	2.47±0.02^{cA}	0.49±0.30^{dB}	-4.39±0.33^{eC}	-8.00±0.08^{eD}	-2.36±0.12^{e}
Extract (75 mg/Kgbw)	4.50±0.30^{bA}	3.75±0.03^{bcA}	2.00±0.30^{cB}	-1.25±0.39^{cC}	2.25±0.14^{c}
Extract (150 mg/Kgbw)	2.78±0.24^{cA}	2.79±0.27^{cA}	-1.77±0.31^{dB}	-4.81±0.24^{dC}	-0.25±0.06^{d}
Extract (300 mg/Kgbw)	2.76±0.26^{cA}	1.00±0.25^{dB}	-4.26±0.31^{eC}	-7.77±0.25^{eD}	-2.07±0.19^{e}

Results are presented as Mean ±SEM for 5 animals. Values that share the same superscript lowercase letter column-wise and those with same uppercase letters row-wise are statistically similar ($p>0.01$; one-factor ANOVA and Tukey's post hoc).

In comparison, the abdominal circumferences in obese mice treated with *P. thonningii* and *L. eriocalyx* extracts at the three studied dosages were statistically insignificant in the first, second and third weeks of the experiment ($p>0.01$). Similarly, the effects of the two extracts at the dosages of 75 and 150mg/Kgbw were non-significant in abdominal circumferences in the fourth week of the experiment ($p>0.01$). However, the abdominal circumferences in mice that received *P. thonningii* extract dose of 300 mg/Kgbw were significantly lower than those noted in mice ($p<0.01$) treated with *L. eriocalyx* extract at the same dose in the fourth week of the study (Figure 4.5).

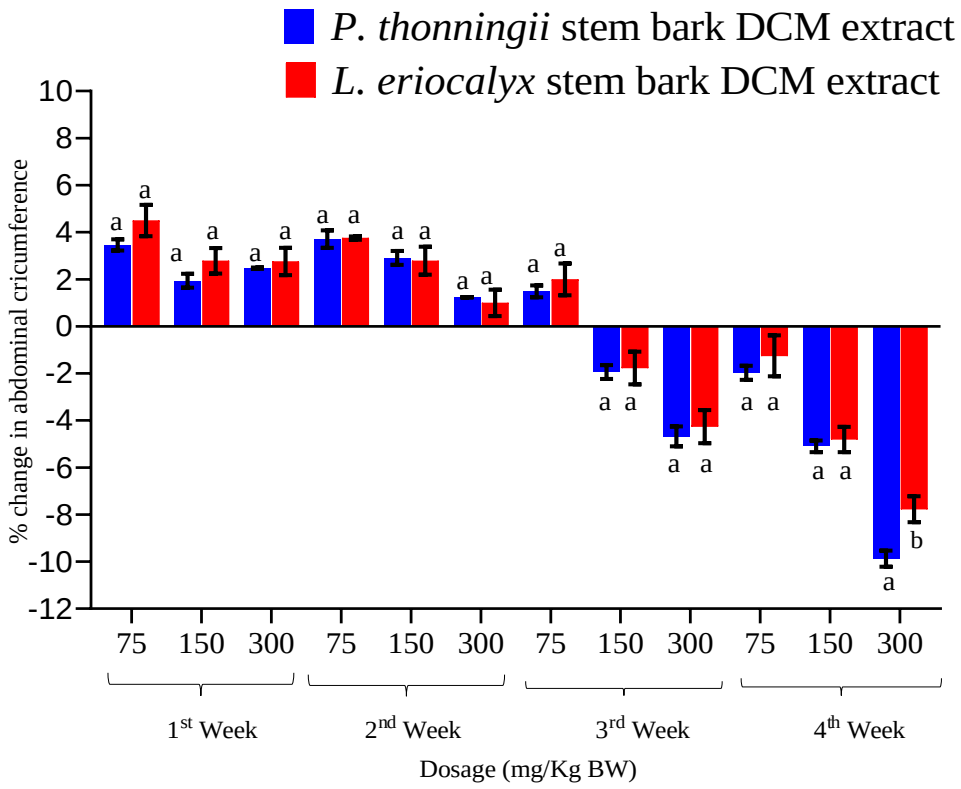


Figure 4.5: Comparison of effects of *Piliostigma thonningii* and *Lonchocarpus eriocalyx* DCM extracts on abdominal circumferences following DMPA-induced obesity in mice.
Bars that share the same letter at the same dosage and week are statistically similar using independent t-test ($p>0.01$).

4.1.6 Effects of *Piliostigma thonningii* and *Lonchocarpus eriocalyx* DCM extracts on Body Adiposity Index (BAI) and Atherogenic Index (AI) in mice following DMPA-induced obesity

Therapy with *P. thonningii* extract resulted in changes in BAI and AI following DMPA-induced obesity in mice (Table 4.11). The BAI and AI of obese control mice were considerably higher than those recorded ($p<0.01$) in mice in the other treatment groups. Therapy with *P. thonningii* extract and Orlistat significantly reduced BAI and AI compared to those noted ($p<0.01$) in obese control mice. (Table 4.11).

The BAI of mice administered with *P. thonningii* extract at the three dosages differed significantly in obese mice ($p<0.01$). However, the effect of Orlistat on BAI statistically matched the effect of the extract ($p>0.01$) at the three studied dosages. In addition, the obese control mice had substantially higher BAI relative to BAI reported in mice ($p<0.01$) of the other treatment groups. Further, the BAI of normal control mice were statistically non-significant compared to BAI noted in ($p>0.01$) Orlistat-treated and extract-treated (doses of 75 and 150 mg/Kgbw) mice (Table 4.11).

The AI of mice treated with the extract at the three dosages differed considerably ($p<0.01$). Nevertheless, the AI noted in positive control mice were statistically insignificant compared to those seen in mice treated with the extract dosages of 75 and 150 mg/Kgbw ($p<0.01$). Besides, the AI in obese control mice were statistically higher relative to those noticed in mice of ($p<0.01$) other treatment groups. Further, the normal control mice had AI that were considerably higher in contrast to those seen ($p<0.01$) in extract-treated mice (Table 4.11).

Table 4.11: Effect of *Piliostigma thonningii* DCM extract on body adiposity index and Atherogenic Index of mice following DMPA-induced obesity

Treatments	BAI (%)	AI (%)
Normal Control	1.63 ± 0.05^{b}	0.05 ± 0.01^{b}
Negative Control	7.03 ± 0.08^{a}	0.55 ± 0.03^{a}
Positive control	1.49 ± 0.05^{bc}	-0.20 ± 0.03^{c}
Extract (75 mg/Kgbw)	1.60 ± 0.03^{b}	-0.16 ± 0.03^{c}
Extract (150 mg/Kgbw)	1.41 ± 0.02^{bc}	-0.27 ± 0.04^{cd}
Extract (300 mg/Kgbw)	1.31 ± 0.02^{c}	-0.38 ± 0.02^{d}

Results are presented as Mean ±SEM for 5 animals. Values that share the same superscript lowercase letter column-wise are statistically similar ($p>0.01$; one-factor ANOVA and Tukey's post hoc).
BAI = Body Adiposity Index**;** AI = Atherogenic Index.

Moreover, the mice administered with *L. eriocalyx* extract at the three studied dosages demonstrated alterations in BAI and AI following DMPA-induced obesity (Table 4.12). The negative control mice had significantly higher values of BAI and AI relative to those reported in ($p<0.01$) the other treatment groups. Therapy with extract significantly lowered BAI and AI compared to those noted ($p<0.01$) in obese control mice. The extract reduced BAI and AI dose-dependently in obese mice (Table 4.12)

The BAI of mice treated with *L. eriocalyx* extract dosages of 75 and 150 mg/Kgbw were statistically non-significant and matched the BAI of ($p>0.01$) normal control and positive control mice. Similarly, the effect of Orlistat on BAI statistically matched the effect of the extract at the three studied dosages ($p>0.01$). However, the BAI of obese control mice were considerably higher in contrast to BAI ($p<0.01$) of mice in the other treatment groups (Table 4.12).

The AI of mice administered with extract at the three dosages differed significantly ($p<0.01$). However, the AI of Orlistat-treated mice were statistically insignificant

compared with AI reported in extract-treated mice at dosages of 75, 150 and 300 mg/Kgbw ($p>0.01$). However, negative control mice exhibited considerably higher AI compared to the AI of mice recorded ($p<0.01$) in other treatment groups (Table 4.12).

Table 4.12: Effect of *Lonchocarpus eriocalyx* DCM extract on body adiposity index and Atherogenic Index of mice following DMPA-induced obesity

Treatment group	BAI (%)	AI (%)
Normal Control	1.63 ± 0.05^{b}	0.05 ± 0.01^{b}
Negative Control	7.03 ± 0.08^{a}	0.55 ± 0.03^{a}
Positive Control	1.49 ± 0.05^{bc}	-0.20 ± 0.03^{cd}
Extract (75 mg/Kgbw)	1.68 ± 0.04^{b}	-0.11 ± 0.03^{c}
Extract (150 mg/Kgbw)	1.54 ± 0.03^{bc}	-0.19 ± 0.02^{cd}
Extract (300 mg/Kgbw)	1.36 ± 0.05^{c}	-0.26 ± 0.02^{d}

Results are presented as Mean ±SEM for 5 animals. Values that share the same superscript lowercase letter column-wise are statistically similar ($p>0.01$; one-factor ANOVA and Tukey's post hoc).
BAI = Body Adiposity Index**;** AI = Atherogenic Index.

In comparison, the BAI of mice treated with the two extracts dosages of 75 and 300 mg/Kgbw were non-significant at the corresponding dosage ($p>0.01$). Nevertheless, the BAI of mice that received *P. thonningii* extract dosage of 150 mg/Kgbw were considerably lower relative to BAI noted in mice treated with *L. eriocalyx* extract ($p<0.01$) at the exact dosage (Figure 4.6).

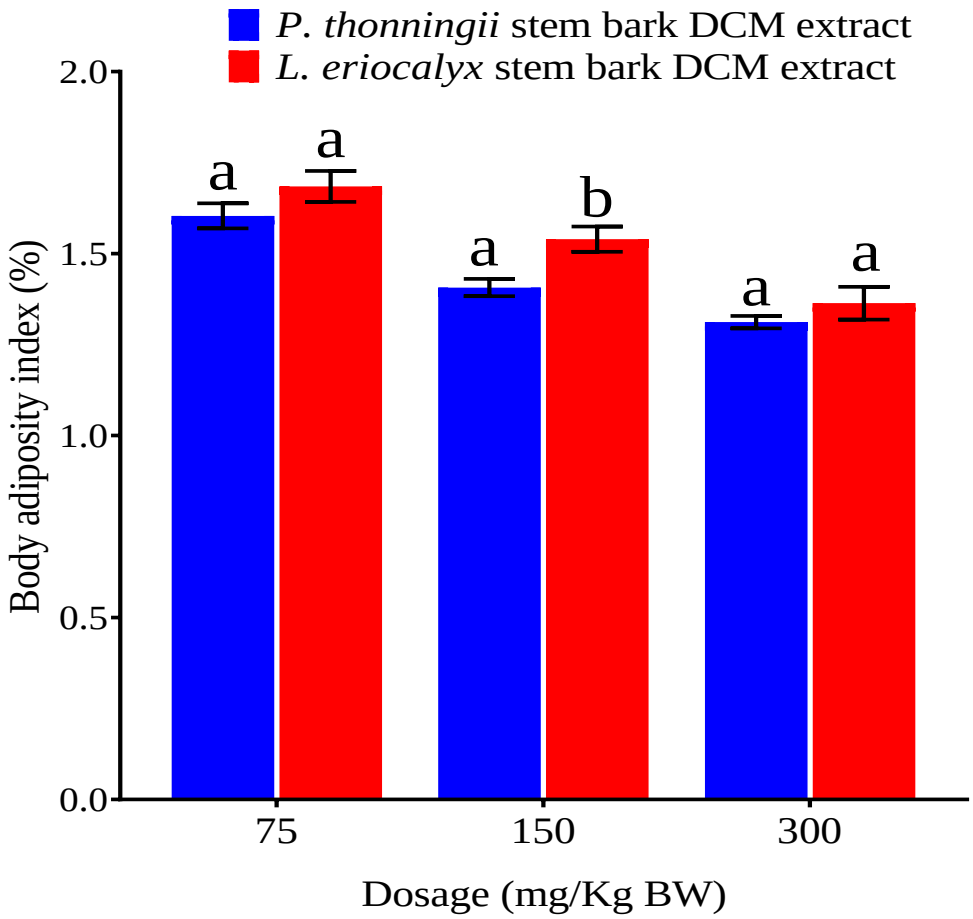


Figure 4.6: Comparison of effects of *Piliostigma thonningii* and *Lonchocarpus eriocalyx* DCM extracts on body adiposity index following DMPA-induced obesity in mice.
Bars that share the same letter at the same dosage are statistically similar using independent ($p>0.01$).

The AI of mice treated with two extracts dosages of 75 and 150 mg/Kgbw were non-significant ($p>0.01$). Nevertheless, the mice treated with *P. thonningii* extract dosage of 300 mg/Kgbw had considerably lower AI relative to AI reported in mice treated with *L. eriocalyx* extract ($p<0.01$) at the equivalent dosage (Figure 4.7).

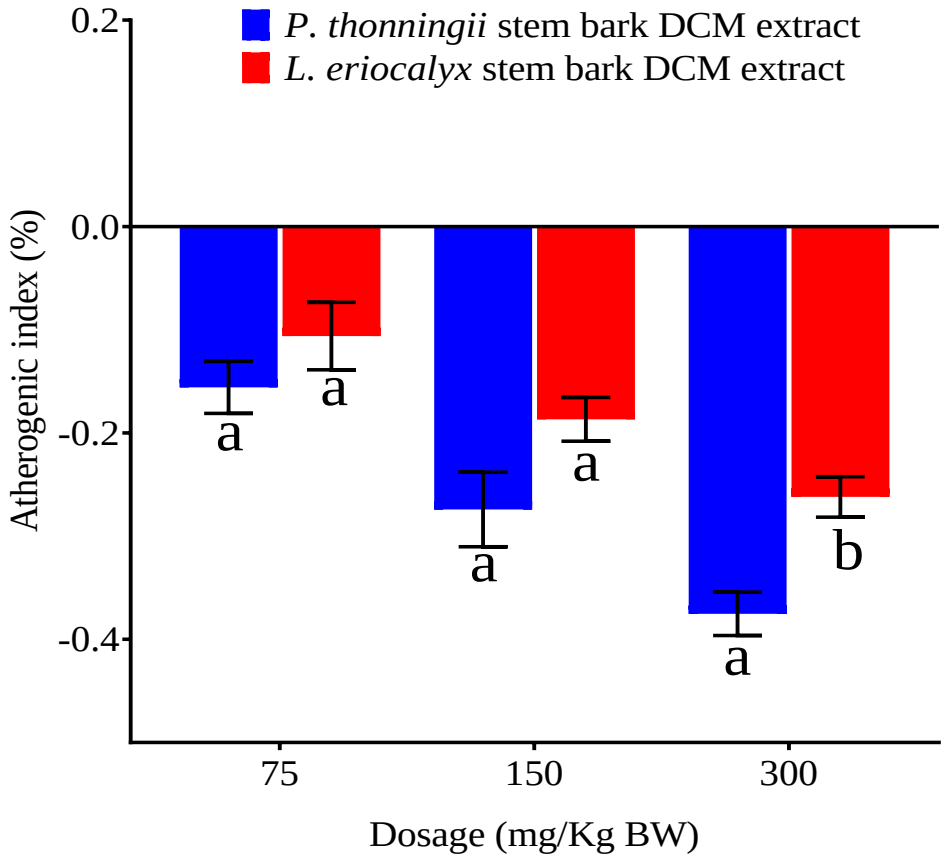


Figure 4.7: Comparison of effects of *Piliostigma thonningii* and *Lonchocarpus eriocalyx* DCM extracts on atherogenic index following DMPA-induced obesity in mice.

Bars that share the same letter at the same dosage are statistically similar using independent t-test ($p>0.01$).

4.2 Effects of *Piliostigma thonningii* and *Lonchocarpus eriocalyx* DCM extracts on the lipid profiles and random blood glucose levels of DMPA-induced obese mice

Therapy with *P. thonningii* extract caused changes in serum lipid and glucose profiles following DMPA-induced obesity in mice (Table 4.13). The negative control mice had significantly high levels of triglycerides (TG), total cholesterol (TC), very-Low-density lipoprotein cholesterol (VLDL-C), low-density lipoprotein cholesterol (LDL-C), and blood glucose, as well as considerably lower levels of high-density lipoprotein cholesterol (HDL-C) (Table 4.13). The Orlistat-treated and extract-treated mice had considerably lower levels of TC, TG, LDL-C, VLDL-C and blood glucose, as well as substantially higher HDL-C levels relative to levels noted ($p<0.01$) in obese control

mice (Table 4.13).

The effect of *P. thonningii* extract dosages of 75, 150 and 300 mg/Kgbw had no significant variations in TC, TG and VLDL-C levels ($p>0.01$) in obese mice. Nevertheless, the effect of the *P. thonningii* extract at the three studied dosages in LDL-C, HDL-C and blood glucose levels differed significantly ($p<0.01$). In addition, the TG, TC, LDL-C and VLDL-C levels in Orlistat-treated mice statistically matched levels in extract-treated mice at the three studied doses ($p>0.01$). Similarly, the HDL-C and blood glucose levels in Orlistat-treated mice were non-significant compared to levels noted in extract-treated mice ($p>0.01$) at the dosage of 75 and 150 mg/Kgbw (Table 4.13). The levels of TG, TC, LDL-C, VLDL-C and blood glucose were significantly higher, while the levels of HDL-C were considerably lower in obese control mice relative to levels recorded ($p<0.01$) in the other treament groups (Table 4.13).

Table 4.13: Effect of *Piliostigma thonningii* DCM extract on lipid profile and random blood glucose levels of mice following DMPA-induced obesity

Treatment group	Lipid profiles mol/L)					Blood glucose (mg/dl)
	TC	TG	HDL-C	LDL-C	VLDL-C	
Normal Control	1.68±0.04^{b}	1.54±0.05^{b}	1.36±0.05^{c}	0.66±0.02^{bc}	0.31±0.01^{b}	103.40±1.94^{c}
Negative Control	2.72±0.07^{a}	2.38±0.08^{a}	0.68±0.06^{d}	1.80±0.05^{a}	0.48±0.02^{a}	200.60±2.75^{a}
Positive control	1.54±0.05^{b}	1.02±0.06^{c}	1.62±0.07^{bc}	0.62±0.04^{bc}	0.20±0.01^{c}	119.80±1.53^{b}
Extract (75 mg/Kg BW)	1.58±0.04^{b}	1.08±0.06^{c}	1.54±0.05^{bc}	0.76±0.05^{b}	0.22±0.01^{c}	128.80±1.77^{b}
Extract (150 mg/Kg BW)	1.54±0.04^{b}	0.94±0.05^{c}	1.76±0.05^{ab}	0.54±0.05^{bc}	0.18±0.01^{c}	118.60±1.94^{b}
Extract (300 mg/Kg BW)	1.44±0.04^{b}	0.82±0.04^{c}	1.94±0.05^{a}	0.52±0.04^{c}	0.16±0.01^{c}	101.00±1.52^{c}

Results are presented as Mean ±SEM for 5 animals. Values that share the same superscript lowercase letter column-wise are statistically similar ($p>0.01$; one-factor ANOVA and Tukey's post hoc).

TG = Triglycerides; TC = Total Cholesterol; LDL-C = Low-density Lipoprotein Cholesterol; VLDL-C = Very-Low-density Lipoprotein Cholesterol; HDL-C = High-density Lipoprotein Cholesterol.

On the other hand, treatment with *L. eriocalyx* DCM extract at the dosages of 75, 150 and 300 mg/Kgbw resulted in alterations in lipid profiles and blood glucose levels following DPMA-induced obesity (Table 4.14). The TG, TC, LDL-C, VLDL-C and blood glucose levels in obese control mice were considerably greater, whereas HDL-C levels were substantially lower compared to levels in ($p<0.01$) the other treatment groups (Table 4.13). Therapy with *L. eriocalyx* extract alleviated abnormal lipid profiles and blood glucose levels close to normal control ranges in obese mice (Table 4.14).

The TG, LDL-C, VLDL-C and HDL-C levels in mice that received *L. eriocalyx* extract dosages of 75, 150 and 300 mg/Kgbw were non-significant ($p>0.01$). However, the TC and blood glucose levels in extract-treated mice differed significantly ($p<0.01$). In addition, the effect of Orlistat on TC, TG, LDL-C, VLDL-C and HDL-C levels statistically matched the effect of the extract ($p>0.01$) at the three studied dosages. Similarly, the levels of blood glucose in Orlistat-treated mice were statistically insignificant compared to those noted in extract-treated mice ($p>0.01$) at the dosages of 75 and 150 mg/Kgbw (Table 4.14).

Table 4.14: Effect of *Lonchocarpus eriocalyx* DCM extract on the lipid and random blood glucose profiles of mice following DMPA-induced obesity

Treatment group	Lipid profiles (mmol/L)					Blood glucose (mg/dl)
	TC	TG	HDL-C	LDL-C	VLDL-C	
Normal Control	1.68±0.04^{bc}	1.54±0.05^{b}	1.36±0.05^{b}	0.66±0.02^{b}	0.31±0.01^{b}	103.40±1.94^{c}
Negative Control	2.72±0.07^{a}	2.38±0.08^{a}	0.68±0.06^{c}	1.80±0.05^{a}	0.48±0.02^{a}	200.60±2.75^{a}
Positive Control	1.54±0.05^{bc}	1.02±0.06^{c}	1.62±0.07^{ab}	0.62±0.04^{b}	0.20±0.01^{c}	119.80±1.53^{b}
Extract (75 mg/Kg bw)	1.78±0.06^{b}	1.16±0.05^{c}	1.48±0.06^{ab}	0.64±0.05^{b}	0.23±0.01^{c}	128.90±1.83^{b}
Extract (150 mg/Kg bw)	1.52±0.04^{c}	1.04±0.04^{c}	1.60±0.07^{ab}	0.50±0.03^{b}	0.21±0.01^{c}	118.60±1.89^{b}
Extract (300 mg/Kg bw)	1.46±0.02^{c}	0.94±0.02^{c}	1.72±0.06^{a}	0.46±0.02^{b}	0.19±0.01^{c}	102.40±2.79^{c}

Results are presented as Mean ±SEM for 5 animals. Values that share the same superscript lowercase letter column-wise are statistically similar ($p>0.01$; one-factor ANOVA and Tukey's post hoc).

TG = Triglycerides; TC = Total Cholesterol; LDL-C = Low-density Lipoprotein Cholesterol; VLDL-C = Very-Low-density Lipoprotein Cholesterol; HDL-C = High-density Lipoprotein Cholesterol.

In comparison, there was no significant variation on lipid profiles in mice administered with *P. thonningii* and *L. eriocalyx* extracts at each tested dosage ($p>0.01$; Figure 4.8).

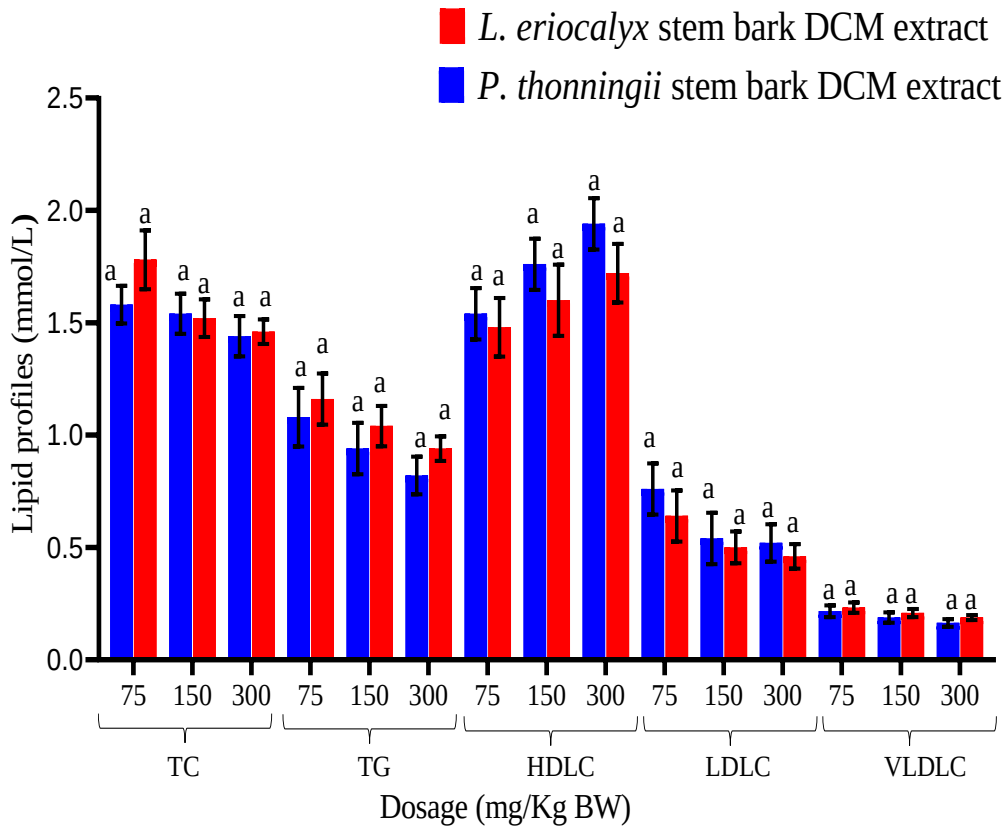


Figure 4.8: Comparison of effects of *Piliostigma thonningii* and *Lonchocarpus eriocalyx* DCM extracts on lipid profiles following DMPA-induced obesity in mice.

Bars that share the same letter at the same dosage are statistically similar using independent t-test ($p>0.01$). TG = Triglycerides; TC = Total cholesterol; VLDL-C = Very-Low-High Density Lipoprotein Cholesterol; LDL-C = Low-density Lipoprotein Cholesterol; HDL-C = High-density Lipoprotein Cholesterol.

Moreover, upon comparison, the blood sugar concentration in mice that received the two tested extracts at each of the three doses were statistically similar ($p>0.01$; Figure 4.9).

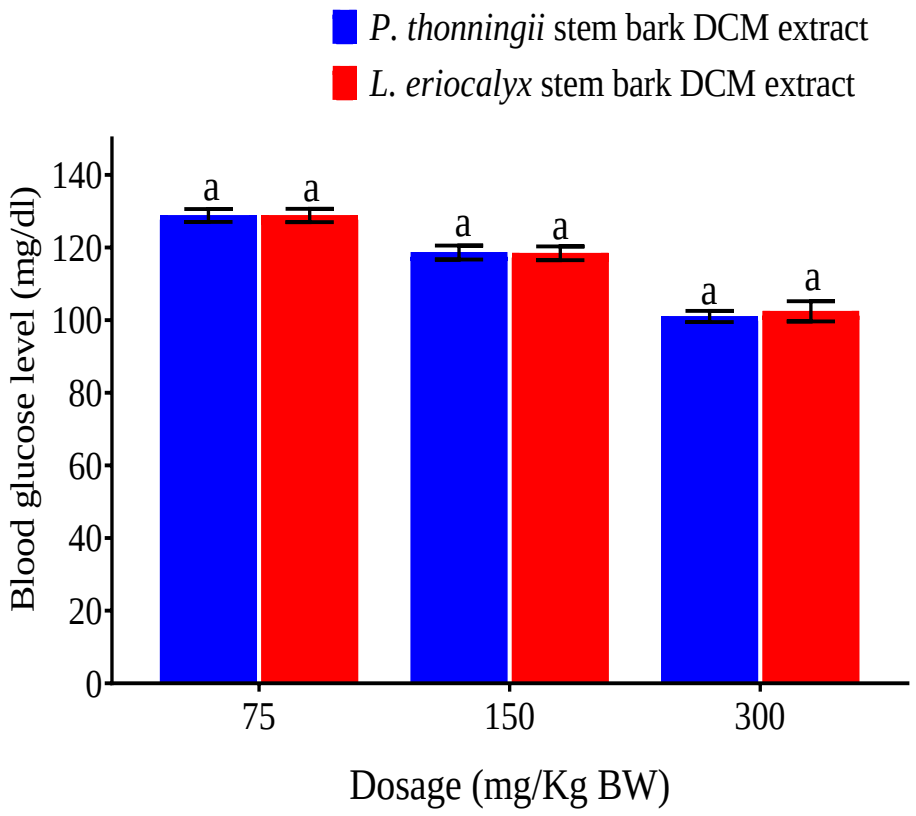


Figure 4.9: Comparison of effects of *Piliostigma thonningii* and *Lonchocarpus eriocalyx* DCM extracts on blood glucose levels following DMPA-induced obesity in mice.
Bars that share the same letter at the same dosage are statistically similar using independent t-test (p>0.01).

4.3 Quantitative phytochemical profiles of *Piliostigma thonningii* and *Lonchocarpus eriocalyx* DCM extracts

Phytochemical analysis showed that *P. thonningii* and *L. eriocalyx* DCM extracts possessed secondary metabolites associated with anti-obesity effects (Table 4.15). The extracts contained phytocompounds of different classes, including fatty acids, hydrocarbons, triterpenoids, alcohols, phytosterols, triglycerides, and phenols. The most abundant secondary metabolites identified in *P. thonningii* included the oleic acid (218.60±15.6ng/g) and 1-nonadecene (33.34±2.38ng/g) and squalene (31.45±2.25 ng/g). The least abundant compounds comprised carvacrol, valencene, and α-cedrene at concentrations of 1.37±0.10 ng/g, 0.72±0.05 ng/g and 0.69±0.05 ng/g, respectively.

Further, the most abundant phytoconstituents in *L. eriocalyx* were gamma-sitosterol followed by taraxasterol and betaamyrin at concentrations of 16.72±1.19ng/g, 14.17±1.01 ng/g and 13.61±0.97 ng/g, correspondingly. The least abundant phytochemicals in the extract of *L. eriocalyx* were 4-(2,4,4-trimethylpentan-2-yl) phenol (0.27±0.02ng/g), methylstearate (0.88±0.06ng/g), and dodecanoicacid,1,2,3-propanetriyl ester (2.55±0.18 ng/g).GC-MS chromatograms of this two plants are available in the appendix section I and II

Table 4.15: Phytochemical profile of *Piliostigma thonningii* and *Lonchocarpus eriocalyx* DCM extracts

Compound name	Molecular formula	Chemical class	Concentration (ng/g)	
			P. thoningii	***L. eriocalyx***
Oleic Acid	$C_{18}H_{34}O_2$	Fatty acid	218.60±15.6	-
1-Nonadecene	$C_{19}H_{38}$	Hydrocarbon	33.34±2.38	-
Squalene	$C_{30}H_{50}$	Triterpene	31.45±2.25	-
Alpha Amyrin	$C_{30}H_{50}O$	Triterpenoid	28.88±2.06	-
Decosanoic acid	$C_{22}H_{44}O_2$	Fatty acid	23.61±1.69	-
Hexadecanol	$C_{16}H_{34}O$	Alcohol	23.50±1.68	-
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Fatty acid	21.88±1.56	5.88±0.42
1-Hexadecene	$C_{16}H_{32}$	Hydrocarbon	18.22±1.30	5.43±0.39
Octacosanol	$C_{28}H_{58}O$	Alcohol	12.47±0.89	-
Gamma Tocopherol	$C_{28}H_{48}O_2$	Phenolic	9.52±0.68	-
Tetradecene	$C_{14}H_{28}$	Hydrocarbon	9.33±0.67	-
Gamma Sitosterol	$C_{29}H_{50}O$	Phytosterol	5.85±0.42	16.72±1.19
Campesterol	$C_{28}H_{48}O$	Phytosterol	5.50±0.39	-
Palmitoleic acid	$C_{16}H_{30}O_2$	Fatty acid	4.28±0.306	-
4-(2,4,4-trimethylpentan-2-yl)phenol	$C_{14}H_{22}O$	Phenolic	2.38±0.17	0.27±0.02
Pentadecanoic acid	$C_{15}H_{30}O_2$	Fatty acid	2.27±0.162	-
Stigmasterol	$C_{29}H_{48}O$	Phytosterol	1.49±0.12	9.12±0.65
Carvacrol	$C_{10}H_{14}O$	Phenolic	1.37±0.10	-
Valencene	$C_{15}H_{24}$	Sesquiterpene	0.72±0.05	-
α-cedrene	$C_{15}H_{24}$	Sesquiterpene	0.69±0.05	-
2-methyl-1-Hexadecanol	$C_{17}H_{36}O$	Alcohol	-	4.05±0.29
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	Fatty acid	-	5.88±0.42
Methyl stearate	$C_{19}H_{38}O_2$	Fatty acid	-	0.88±0.06
1-Triacontanol	$C_{30}H_{62}O$	Alcohol	-	8.20±0.59
Dodecanoic acid, 1,2,3-propanetriyl ester	$C_{39}H_{74}O_6$	Triglyceride	-	2.55±0.18
Ergost-5-en-3-ol, (3 beta, 24R)	$C_{28}H_{48}O$	Phytosterol	-	4.04±0.29
Beta Amyrin	$C_{30}H_{50}O$	Triterpenoid	-	13.61±0.97
Taraxasterol	$C_{30}H_{50}O$	Triterpenoid	-	14.17±1.01
9 octadecenoic acid	$C_{18}H_{34}O_2$	Fatty acid	-	4.07±0.29

CHAPTER FIVE

DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

5.1 Discussion

Globally, the burden of obesity is increasing due to various contributing factors, such as diet, genetic predisposition, sedentary lifestyle, psychological and physiological factors as well as endocrine disorders, among others (Dai *et al*., 2020). As a result, there is heavy financial expenditure on treating and managing obesity and associated health complications, with unbearable burdens in developing nations, particularly those in sub-Saharan Africa (Seidell and Halberstadt, 2015). Moreover, obesity leads to a plethora of metabolic, psychological, and cardiovascular diseases, among others, and significantly reduces the life expectancy of the affected persons (Gadde *et al*., 2018a).

Although various pharmacological agents have been investigated and valorised to treat obesity in affected patients, only a few have been approved for clinical application (Lin and Li, 2021). The main anti-obesity drugs, Orlistat and Sibutramine, are administered to affect lipid mobilisation in the body, thus averting weight gain. However, these drugs cause adverse side effects, which limit their usability (Kim *et al*., 2014; Gadde *et al*., 2018b). Consequently, alternative stratagems, especially through empirical investigation of promising medical plants, may provide efficacious, safe, and affordable anti-obesity therapies (Gooda *et al*., 2012). Accordingly, the current research investigated the activities of anti-obesity and quantitative phytochemistry of *L. eriocalyx* and *P. thonningii* DCM extracts as promising leads of effective therapies for obesity.

At cellular level, obesity is characterised by hyperplasia and hypertrophy of differentiated adipocytes in the adipose tissue, leading to significant increase or elevation of key anthropometric and biochemical parameters (Thomas *et al*., 2018). Anthropometrics is the best measure and predictor of central obesity and augment the diagnosis of metabolic syndromes (Piqueras *et al*., 2021). Impaired lipid and energy utilization in the body lead to considerable increase in body and organ weights, fat pad weights, body mass indices (BMIs), and body adiposity indices (BAIs), among other parameters. Further, somatic indices (body weight and relative organ weight) are important measures for disproportionate lipid deposition due to an imbalance between lipogenesis and lipolysis in the body (Piqueras *et al*., 2021). Excessive lipid deposition on various organs impairs their proper functioning resulting in various maladies and death if not adequately mitigated (Gadde *et al*., 2018b).

Previous research shows that various progesterone-based contraceptives, especially DMPA, induce obesity in women and female rodents (Beksinska *et al*., 2021). In this study, depo-medroxyprogesterone acetate (DMPA) was injected into female experimental mice to induce obesity. Progesterone and its analogues stimulate hyperphagia in the body upon interacting with progestin receptors, which are expressed by the serotonergic neurons. Further, a disturbance of ovarian progesterone levels predisposes females to binge eating, which alters serotonin levels and serotonergic receptor functioning in the body (Sims *et al*., 2020).

The negative control mice, which received DMPA and 1% DMSO (placebo), had significantly higher body weights, relative organ weights, fat pad weights, body mass indices (BMIs), abdominal circumference, body adiposity indices (BAI), and

atherogenic indices (AIs) compared with all the other experimental mice. Elevation of these anthropometric parameters indicates that DMPA, at a dosage of 10 mg/Kgbw, successfully induced obesity in the experimental animals (Antunes *et al*., 2016). These results corroborate those of other research findings, which demonstrated progesterone (DMPA) as the most obesogenic of all the steroidal hormones (Sims *et al*., 2020; Beksinska *et al*., 2021). Thus, a drug agent capable of lowering or maintaining anthropometric parameters of DMPA-induced obese mice at normal levels is a potential and promising anti-obesity drug.

Orlistat, a conventional anti-obesity therapy, was chosen in this study as a reference drug (positive control) based on its demonstrable efficacy and clinical application. Empirical research shows that Orlistat exerts its anti-obesity efficacy by inhibiting pancreatic lipase and interfering with lipid digestion and uptake (Johnson and Schwartz, 2018). Besides, Orlistat has been shown to suppress hyperphagia by modifying monoamine neurotransmitters associated with appetite (Qi, 2018). Moreover, Orlistat slows the reuptake of serotonin (5-HT) at the hypothalamic site and reduces ghrelin hormone, thus regulating food consumption and gain in body weight (Leung *et al*., 2003; Qi, 2018).

All the anthropometric parameters were considerably lower in Orlistat-treated mice than in obese control mice. These results indicate that Orlistat (10 mg/Kgbw) successfully averted DMPA-induced obesity in experimental mice, which agrees with previous research reports. Moreover, it demonstrates a close interaction between the serotonin receptor system and neurosteroids, such as progesterone, whose modulation is vital in averting clinical obesity (Choi *et al*., 2020). Therefore, it is suggestive that a

drug agent demonstrating similar effects to Orlistat may be a potential anti-obesity therapy.

L. eriocalyx and *P. thonningii* DCM extracts significantly decreased the investigated anthropometric parameters in DMPA-induced dose-dependently across the experimental period. Notably, the anthropometric parameters of the experimental mice treated with *P. thonningii* extract dosages of 150 and 300 mg/Kgbw, as well as *L. erocalyx* DCM extract dose of 300 mg/Kgbw were comparable with those of the positive control mice throughout the study period, depicting higher anti-obesity efficacy. Besides, at all doses, the two extracts did not upset the relative organ weights of experimental mice, which was consistent with the measured body weights, demonstrating their efficacy in ameliorating DMPA-induced lipid deposition in the body. The findings herein correspond with other scholar, who previously reported significant anti-obesity efficacy of various plant extracts in obesity mice models (D'Mello *et al*., 2011).

The effects of the two DCM extracts in averting increase in various anthropometric measures in obese mice can be attributed to various bioactive phytochemicals, which may have exerted their efficacy solely or synergistically (Kim *et al*., 2015; Azzini *et al*., 2017). These plant-derived amalgams may have reduced excessive hunger and appetite for food, thus maintaining energy homeostasis (Kelleni, 2018; Kumar *et al*., 2022; Nonogaki, 2022). Besides, these phytochemicals may have conferred the studied plant extracts' anti-obesity efficacy by modifying the satiety signalling cascade, inhibiting pancreatic lipase, and modulating serotonin levels and function to avert DMPA-induced effects (Dincer and Yuksel, 2021; Saad *et al*., 2021).

Extensive research has established characteristically abnormal lipid profile in obese population (Azmi *et al*., 2021; Hossain *et al*., 2021). Upregulated visceral adiposity causes lipotoxicity, associated with elevated levels ot TC, TG, LDL-C, VLDL-C, and low levels of HDL-C, which are hallmarks of dyslipidemia (Azmi *et al*., 2021). Dyslipidemia is linked to emergency and exacerbation of other metabolic syndromes like diabetes mellitus, inflammation, cardiovascular disorders, as well as other chronic conditions with debilitating sequelae (Vekic *et al*., 2019; Shabana *et al*., 2020).

Hypertrophic and hyperplasic adipocytes promote excessive lipogenesis and secretion of the free fatty acids, their processing inside the liver, and subsequent modifications to VLDL and LDL (Vekic *et al*., 2019). Disproportionate accumulation of VLDL, LDL, and associated remnants evokes metabolic insult and morphological aberrations of organs and internal structures, leading to undesirable consequences (Begum *et al*., 2019; Willey *et al*., 2014). Owing to lipid profiles significance in diagnosing and treating obesity and associated syndromes, this study investigated the effects of the tested extracts to appraise their anti-obesity efficacy. It was found that the negative control mice had substantially higher levels of TG, LDL-C, VLDL-C, and lower HDL-C levels than in the other experimental group mice. These results depict the detrimental role DMPA in interfering with the normal lipid profile, leading to various complications, such as impaired glycemic control, metabolic syndrome, preeclampsia, among others observed in obese individuals (Fekadie, 2015; Article *et al*.,2018; Asare *et al*., 2021). Consequently, a drug agent, which can remodel lipid metabolism while maintaining the normal profile, may be a potential anti-obesity therapy.

The reference drug (Orlistat) significantly reduced the TG, VLDL-C and LDL-C levels but significantly increased HDL-C levels in mice, indicating crucial role of lipid homeostasis in averting obesity. It is apparent that HDL-C helps reduce the VLDL-C and LDL-C in the body, arresting their adverse effects, including inflammation and associated syndromes (Bonizzi *et al*., 2021; Tang *et al*., 2021). Similarly, lipid profiles of mice treated with the studied extracts were comparable with the levels of the positive and normal control groups regardless of dosage. These findings partly suggest that the studied plant extract phytoconstituents increased HDL-C and either downregulated the production or increased the catabolism of VLDL-C and LDL-C in the body of the treated experimental mice (Bordalo, 2018; Santos and Lavie, 2021). Thus, the observed anti-obesity potency of the tested plant extracts may be quite linked to their ability to stabilise lipid concentrations and suppress the elevation of lipotoxic adducts through their bioactive phytochemical amalgams (Ahn and Kim, 2016; Arika *et al*., 2019a; Wang *et al*., 2020).

Lipid and carbohydrate metabolisms are tightly linked through a feedback control mechanism to maintain energy homeostasis (Singh *et al*., 2015). Excessive intake of high-caloric diets yields excess glucose above the body's requirements, as evidenced by elevated serum glucose levels during routine tests (Wang *et al*., 2020). The excess glucose is transformed into lipids, primarily TGs, which are subsequently esterified and modified to other lipoproteins (Abete *et al*., 2010). The produced lipids are secreted into circulation, transported to the adipose tissue for storage, and often accumulate at various somatic sites causing undesired functional and structural anomalies (Vekic *et al*., 2019).

Ordinarily, when the body is fasting, the stored TGs and other associated lipids are mobilised through a lipolytic pathway to liberate their stored energy for cell functioning (Yeh *et al*., 2021). However, this delicate homeostatic feedback mechanism is disrupted by obesogenic agents, which promote lipogenesis, hinder lipolysis, and impair proper metabolic signalling and regulation, resulting in myriad syndromes (Klop *et al*., 2013; Morgan *et al*., 2016).

The levels of blood glucose in normal control and positive control mice as well as those treated with the extracts of *L. eriocalyx* and *P. thonningii* at 150 and 300 mg/Kgbw, were comparable. However, the obese control mice showed substantial increase in blood glucose levels compared to levels of the other treatment group mice. Moreover, the levels of blood glucose in mice administered with the extracts at all doses were statistically similar. The observations made in this current study reiterate the close connection between lipid and carbohydrate metabolisms and their role in sustaining energy equilibrium in the body (Abete *et al*., 2010; Singh *et al*., 2015; Article and Alawdi, 2018).

Further, these findings are consistent with lipid profiles and anthropometric measures observed in this study and positively corroborate those reported previously (Park *et al*., 2014; Yang *et al*., 2018; Lee *et al*., 2020). The relationship between high caloric diet, body adiposity index and atherogenic index in obesity and associated complications has been demystified (Bano *et al*., 2009; Nedergaard *et al*., 2017; Cho *et al*., 2020). Ultimately, the studied plant extracts contained anti-obesity-associated active principles, which modulated metabolism, attenuating DMPA-induced obesity in

mice (Rivera-Ramírez *et al*., 2011; Jang *et al*., 2013; Huang *et al*., 2014; Saad *et al*., 2021).

The utilisation of medicinal plants as food and medicines has been practised since antiquity, with appreciable potency levels (James *et al*., 2018). A preponderance of empirical evidence reveals that plants synthesise various secondary metabolites in varying quantities, which are responsible for medicinal efficacy (Moriasi *et al*., 2021). These active compounds are produced in varying quantities in response to biotic and abiotic adversity (Kennedy *et al*., 2011; Kurmukov, 2013; Koche *et al*., 2016). The health-promoting advantages of these plants are conferred to humans and animals when consumed as food and medicine (Moriasi *et al*., 2021).

Irrefutably, the efficacious medicines or their analogues and prodrugs used in conventional medicine were derived from medicinal plants (Greenwell *et al*., 2015; Jamshidi-Kia *et al*., 2018). Nevertheless, despite widespread utilization of medicinal plants to treat various diseases, including obesity, metabolic syndromes, inflammation, cancer, and neurological disorders, among other devastating diseases, only a small proportion have been investigated and validated empirically (Ben *et al*., 2020; Akimat *et al*., 2021; Halim *et al*., 2022).

Besides, bioactive components of most medicinal plants, including *P. thonningii* and *L. eriocalyx*, especially those responsible for their anti-obesity efficacy, have not been deciphered. Accordingly, quantitative phytochemical analyses of *L. eriocalyx* and *P. thonningii* DCM extracts were performed in this present study to determine the probable phytocompounds responsible for anti-obesity efficacy.

Previous research shows that flavonoids, alkaloids, phenols, phytosterols, and terpenoids are responsible for anti-obesity efficacy of herbal plants (Arika *et al*., 2019b; Moriasi *et al*., 2020a). The quantitative phytochemistry of these studied extracts revealed varied concentrations of various anti- obesity-associated bioactive amalgams. Notably, the extract of *P. thonningii* was richly endowed with fatty acids, terpenoids (triterpenes and sesquiterpenes), phenols and phytosterols, among others. Besides, extract of *L. eriocalyx* contained high amounts of phytosterols, fatty acids, and triterpenoids. The anti-obesity efficacy of these two plant extracts observed in this present study was attributed to these phytochemicals (Zhang *et al*., 2015; Pan *et al*., 2018; Moriasi *et al*., 2020b).

Research studies have elucidated various mechanisms through which specific phytocompounds mediate their anti-obesity efficacy (Pan *et al*., 2018; Zhang *et al*., 2015). Plant-derived fatty acids and phytosterols inhibit the accumulation of triglycerides in the body and suppress lipogenic genes expression. As a result, these effects impede the excessive synthesis and accumulation of lipids in the body, a characteristic feature of obesity. Thus, fatty acids like oleic acid and phytosterols such as stigmasterol, sitosterol, and campesterol may have downregulated the expression and activity of lipogenic genes in DMPA-induced mice to thwart the obesogenic phenotype.

Moreover, plant-derived fatty acids and phytosterols can thwart dyslipidemia, especially by increasing HDL-C levels while reducing LDL-C, VLDL-C and their adducts (Islam *et al*., 2021). Therefore, these compounds probably contributed

to energy balance re-modelling and ameliorating DMPA-induced obesity in mice. Additionally, Poly- and monosaturated fatty acids increase the secretion of cholecystokinin, and other satiety hormones, thereby controlling food intake and appetite (Arika *et al*., 2019a; Kumar *et al*., 2022; Nonogaki, 2022). Thus, the high amounts of decosanoic acid, oleic acid, hexadecenoic acid, palmitoleic, and methyl stearate in this plant extracts under study may be partly involved in the observed anti-obesity efficacy in mice (Kumar *et al*., 2022).

This study also revealed considerable amounts of terpenes, terpenoids, and sesquiterpenes in the studied plant extracts. These phytocompounds have been shown to exert their anti-obesity efficacy by enhancing anti-obesity properties of leptin a well as suppressing the hypothalamic protein tyrosine kinase 1B (Afonso *et al*., 2021; Kim *et al*., 2022). Thus, squalene, α-amyrin, valencene, α-cedrene in the extract of *P. thonningii* as well as β-amyrin and taraxasterol contained in the extracts of *L. eriocalyx* spontaneously played crucial roles in averting DMPA-induced obesity in experimental mice.

This study also revealed the presence of phenols in this plant extracts under study. Previous reports indicate that phenols exhibit hypolipidemic effects and normalise a dysregulated lipid profile in the body (Rodríguez-Pérez *et al*., 2019). In addition, phenols have been shown to suppress adipocyte differentiation, thereby limiting fat deposition in the adipose tissue and other body sites, thus deterring obesogenic traits (Thomas *et al*., 2018). Thus, the presence of carvacol and γ-tocopherol in the extract of *P. thonningii* and 4-(2,4,4-trimethylpentan-2-yl)-phenol in the two extracts fostered the anti-obesity efficacy (Lee *et al*., 2021; Eseberri *et al*., 2022).

The differences in efficacy of *L. eriocalyx* and *P. thonningii* DCM extracts which were evident in this current study, may be attributable to the differences in type and concentrations of bioactive phytochemical amalgams. Besides, research shows that multiple combinations of various phytocompounds may cause synergistic bioactivity, which may be mediated through multiple targets (Rodríguez-Pérez *et al*., 2019; Jaradat *et al*., 2022). Conversely, some phytochemicals may antagonise others, thereby leading to reduced efficacy, which may partly explain the observations made in this present study.

Despite *P. thonningii* extract displaying many phytochemicals in higher concentrations than those observed in the extract of *L. eriocalyx*, their anti-obesity efficacies were generally comparable. Further empirical investigations are encouraged to elucidate the mechanisms by which the studied plant extracts exert their anti-obesity efficacy. Perhaps, the anti-obesity activity of these phytochemicals may have been executed via different and specific targets, which may be related or otherwise (Klop *et al*., 2013; Kim *et al*., 2014).

Previously, pharmacological activity of methanolic and aqueous extracts of *L. eriocalyx* and *P. thonningii* stem barks against other obesity-related diseases have been demonstrated (Olela *et al*., 2020; Moriasi *et al*., 2021)

5.2 Conclusions

Based on the study's findings, the ensuing conclusions were made.

i. The DCM stem extracts of *Piliostigma thonningii* and *Lonchocarpus eriocalyx* possessed anti-obesity activities that lowered anthropometric

parameters following DMPA-induced obesity in mice.

ii. The DCM stem extracts of *Piliostigma thonningii* and *Lonchocarpus eriocalyx* ameliorated abnormal lipid profiles following DMPA-induced obesity in mice.

iii. The DCM stem extracts of *Piliostigma thonningii* and *Lonchocarpus eriocalyx* contained phytochemicals associated with anti-obesity effects.

5.3 Recommendations

5.3.1 Recommendations from the Current Studys

i. The DCM stem extracts of *Piliostigma thonningii* and *Lonchocarpus eriocalyx* can be utilized as potential leads in developing anti-obestity agents which are efficacious and affordable.

ii. The phytochemicals of DCM stem extracts of *Piliostigma thonningii* and *Lonchocarpus eriocalyx* have beneficial phytocompounds that can be harnessed in treating anti-obesity.

5.3.2 Recommendations for further study

i. Further isolate and characterize the quantified phytocompounds in the studied plant extracts to determine their contribution to anti-obesity efficacy and elucidate their specific modes of action.

ii. Considering that this study only focused on the stem bark extracts of the 2 plants, further anti-obesity investigations on other parts within the plant may provide crucial data to appraise their potential in treating obesity.

iii. Toxicity studies of the *Piliostigma thonningii* and *Lonchocarpus eriocalyx* DCM stem extracts should be investigated to evaluate their safety.

REFERENCES

Abete, I., Astrup, A., Martínez, J. A., Thorsdottir, I., and Zulet, M. A. (2010). Obesity and the metabolic syndrome: role of different dietary macronutrient distribution patterns and specific nutritional components on weight loss and maintenance. *Nutrition Reviews, 68*(4), 214-231.

Afolayan, M., Srivedavyasasri, R., Asekun, O. T., Familoni, O. B., Orishadipe, A., Zulfiqar, F., and Ross, S. A. (2018). Phytochemical study of *Piliostigma thonningii*, a medicinal plant grown in Nigeria. *Medicinal Chemistry Research, 27*, 2325-2330.

Afonso, A. F., Pereira, O. R., and Cardoso, S. M. (2021). Salvia species as nutraceuticals: Focus on antioxidant, antidiabetic and anti-obesity properties. *Applied Sciences, 11*(20), 9365.

Ahn, N., and Kim, K. (2016). High-density lipoprotein cholesterol (HDL-C) in cardiovascular disease: effect of exercise training. *Integrative Medicine Research, 5*(3), 212-215.

Akimat, E. K., Omwenga, G. I., Moriasi, G. A., and Ngugi, M. P. (2021). Antioxidant, anti-inflammatory, acute oral toxicity, and qualitative phytochemistry of the aqueous root extract *of Launaea cornuta* (hochst. Ex oliv. and hiern.). *Journal of Evidence-Based Integrative Medicine, 26*, 2515690X211064585.

Aktar, N., Qureshi, N. K., and Ferdous, H. S. (2017). Obesity: a review of pathogenesis and management strategies in adult. *Delta Medical College Journal, 5*(1), 35-48.

Albaik, M., Khan, J., Ardawi, M. S., and Moselhy, S. S. (2016). Predication of biochemical changes in obese women. *International. Journal. Life Sci. Res., 4*, 220-226.

Antunes, K. A., Baldivia, D. D. S., da Rocha, P. D. S., Casagrande, J. C., Argandoña, E. J. S., Vieira, M. D. C., and de Picoli Souza, K. (2016). Antiobesity effects of hydroethanolic extract of *Jacaranda decurrens* leaves. *Evidence-Based Complementary and Alternative Medicine, 2016*.

Arika, W. M., Kibiti, C. M., Njagi, J. M., and Ngugi, M. P. (2019a). Effects of DCM leaf extract of *Gnidia glauca* (Fresen) on locomotor activity, anxiety, and exploration-like behaviors in high-fat diet-induced obese rats. *Behavioural Neurology, 2019*.

Arika, W. M., Kibiti, C. M., Njagi, J. M., and Ngugi, M. P. (2019b). Anti-obesity effects of dichloromethane leaf extract of *Gnidia glauca* in high fat diet-induced obese rats. *Heliyon, 5*(11), e02800.

Arseneau, E., Landry, S., and Darling, E. K. (2019). The Polyamorous Childbearing and Birth Experiences Study (POLYBABES): a qualitative study of the health care experiences of polyamorous families during pregnancy and birth. *CMAJ, 191*(41), E1120-E1127.

Asare, L., Asare, G. A., Owiredu, W. K., Obikorang, C., Appiah, E., Tashie, W., and Seidu, L. (2021). The use of hormonal contraceptives and preeclampsia among Ghanaian pregnant women. *Open Journal of Obstetrics and Gynecology, 11*(04), 419.

Atieno Ochung, A., Okinda Owuor, P., Arot Manguro, L., and Ishola, I. (2020). *Lonchocarpus eriocalyx* (Harms) herb extract for use as painkillers.

Azmi, S., Ferdousi, M., Liu, Y., Adam, S., Siahmansur, T., Ponirakis, G., and Soran, H. (2021). The role of abnormalities of lipoproteins and HDL

functionality in small fibre dysfunction in people with severe obesity. *Scientific Reports*, *11*(1), 1-9.

Azzini, E., Giacometti, J., and Russo, G. L. (2017). Antiobesity effects of anthocyanins in preclinical and clinical studies. *Oxidative Medicine and Cellular Longevity*, *2017*.

Babatunde, O. (2017). GC-MS analysis of leaf, stem-bark and root extracts of *Alstonia boonei*. *African Journal of Pharmacy and Pharmacology*, *11*(46), 577-581.

Balakumar, P., Maung-U, K., and Jagadeesh, G. (2016). Prevalence and prevention of cardiovascular disease and diabetes mellitus. *Pharmacological research*, *113*, 600-609.

Bano, F., Wajeeh, M., Baig, N., Naz, H., Akhtar, N., and Hajra, N. (2009). Antiobesity, antihyperlipidemic and hypoglycemic effects of the aqueous extract of Nigella Sativa seeds (Kalongi). *Journal Biochemistry Molecular Biology*, *42*(4), 136-140.

Basu, A., Du, M., Sanchez, K., Leyva, M. J., Betts, N. M., Blevins, S., and Lyons, T. J. (2011). Green tea minimally affects biomarkers of inflammation in obese subjects with metabolic syndrome. *Nutrition*, *27*(2), 206-213.

Begum, A., and Irfan, S. R. (2019). Lipid profile abnormalities in Bangladeshi type 2 diabetic patients attending a tertiary care hospital: A cross-sectional study. *Bangladesh Critical Care Journal*, *7*(1), 44-47.

Beksinska, M., Issema, R., Beesham, I., Lalbahadur, T., Thomas, K., Morrison, C., ... and Smit, J. (2021). Weight change among women using intramuscular depot medroxyprogesterone acetate, a copper intrauterine device, or a levonorgestrel implant for contraception: findings from a randomized, multicentre, open-label trial. *EClinicalMedicine*, *34*, 100800.

Bollapragada, M. K., Shantaram, M. A. N. J. U. L. A., and Kumar, S. U. N. I. L. (2017). Obesity: Development, epidemiology, factors affecting, quantity, health hazards, management and natural treatment-a review. *International Journal Pharmacy Science*, *9*(2), 12-26.

Bonizzi, A., Piuri, G., Corsi, F., Cazzola, R., and Mazzucchelli, S. (2021). HDL dysfunctionality: clinical relevance of quality rather than quantity. *Biomedicines*, *9*(7), 729.

Bordalo, A. D. (2018). Improving HDL Functions-A New Version of the Quest of sancgreal. *Journal of Cardiology and Cardiovascular Sciences*, *2*(2).

Buch, A., Ben-Yehuda, A., Rouach, V., Maier, A. B., Greenman, Y., Izkhakov, E., ... and Eldor, R. (2022). Validation of a multi-frequency bioelectrical impedance analysis device for the assessment of body composition in older adults with type 2 diabetes. *Nutrition and Diabetes*, *12*(1), 45.

Cantó, C., Menzies, K. J., and Auwerx, J. (2015). NAD+ metabolism and the control of energy homeostasis: a balancing act between mitochondria and the nucleus. *Cell metabolism*, *22*(1), 31-53.

Chatterjee, A., Gerdes, M. W., and Martinez, S. G. (2020). Identification of risk factors associated with obesity and overweight—a machine learning overview. *Sensors*, *20*(9), 2734.

Cho, B. O., Choi, J., Kang, H. J., Che, D. N., Shin, J. Y., Kim, J. S., and Jang, S. I. (2020). Anti-obesity effects of a mixed extract containing *Platycodon grandiflorum*, *Apium graveolens* and green tea in high-fat-diet-induced obese mice. *Experimental and Therapeutic Medicine*, *19*(4), 2783-2791.

Choi, W., Moon, J. H., and Kim, H. (2020). Serotonergic regulation of energy

metabolism in peripheral tissues. *Journal of Endocrinology*, *245*(1), R1-R10.

Chowdhury, M. A. B., Adnan, M. M., and Hassan, M. Z. (2018). Trends, prevalence and risk factors of overweight and obesity among women of reproductive age in Bangladesh: a pooled analysis of five national cross-sectional surveys. *BMJ open*, *8*(7), e018468.

Dai, H., Alsalhe, T. A., Chalghaf, N., Riccò, M., Bragazzi, N. L., and Wu, J. (2020). The global burden of disease attributable to high body mass index in 195 countries and territories, 1990–2017: An analysis of the Global Burden of Disease Study. *PLoS medicine*, *17*(7), e1003198.

Dasofunjo, K., Nwodo, O. F. C., Yakubu, O. E., Ejoba, R., Ukpanukpong, R., Ipav, S., ... and Ezugwu, H. C. (2013). Toxicological implication of ethanol leaf extract of *Piliostigma thonningii* on renal function indices of male wistar albino rats. *European Journal of Experimental Biology*, *3*(3), 652-655.

Dincer, Y., and Yuksel, S. (2021). Antiobesity effects of phytochemicals from an epigenetic perspective. *Nutrition*, *84*, 111119.

D'Mello, P. M., Darji, K. K., and Shetgiri, P. P. (2011). Evaluation of antiobesity activity of various plant extracts. *Pharmacognosy Journal*, *3*(21), 56-59.

Edith, M. (2019). Obesity in Kenya: A Synthesis of the Literature. *Biomedical Journal of Scientific and Technical Research*, *22*(1), 16286-16288.

Erhirhie, E. O., Ekene, N. E., and Ajaghaku, D. L. (2014). Guidelines on dosage calculation and stock solution preparation in experimental animals' studies. *Journal of Natural Sciences Research*, *4*(18), 100-106.

Eseberri, I., Trepiana, J., Léniz, A., Gómez-García, I., Carr-Ugarte, H., González, M., and Portillo, M. P. (2022). Variability in the beneficial effects of phenolic compounds: A review. *Nutrients*, *14*(9), 1925.

Fekadie, M. (2015). Effects of Depo-medroxyprogesterone Acetate (DMPA) on Lipid Profile, Body Weight and Blood Pressure among Women in Tekele Hymanot and Lomeda Health Centers, Addis Ababa, Ethiopia (Doctoral dissertation, Addis Ababa University).

Fruh, S. M. (2017). Obesity: Risk factors, complications, and strategies for sustainable long-term weight management. *Journal of the American Association of Nurse Practitioners*, *29*(S1), S3-S14.

Gachanja, A. N., Kareru, P. G., Kenji, G. M., Keriko, J. M., and Mungai, G. (2007). Traditional Medicines Among the Embu and Mbeere Peoples of Kenya. *African Journal Traditional Complementary Alternative Medicine (Online)*, 75-86.

Gadde, K. M., Martin, C. K., Berthoud, H. R., and Heymsfield, S. B. (2018). Obesity: pathophysiology and management. *Journal of the American College of Cardiology*, *71*(1), 69-84.

Gil-Rojas, Y., Garzón, A., Hernández, F., Pacheco, B., González, D., Campos, J., ... and Rosselli, D. (2019). Burden of disease attributable to obesity and overweight in Colombia. *Value in Health Regional Issues*, *20*, 66-72.

Gooda Sahib, N., Saari, N., Ismail, A., Khatib, A., Mahomoodally, F., and Abdul Hamid, A. (2012). Plants' metabolites as potential antiobesity agents. *The Scientific World Journal*, *2012*.

Gouda, H. N., Charlson, F., Sorsdahl, K., Ahmadzada, S., Ferrari, A. J., Erskine, H., ... and Whiteford, H. (2019). Burden of non-communicable diseases in sub-Saharan Africa, 1990–2017: results from the Global Burden of Disease Study 2017. *The Lancet Global Health*, *7*(10), e1375-e1387.

Greenwell, M., and Rahman, P. K. S. M. (2015). Medicinal plants: their use in

anticancer treatment. *International Journal of Pharmaceutical Sciences and Research*, *6*(10), 4103.

Guideline, O. O. (2001). 425: acute oral toxicity—up-and-down procedure. *OECD Guidelines for the Testing of Chemicals*, *2*, 12-16.

Gutiérrez-López, L., Olivares-Corichi, I. M., Martínez-Arellanes, L. Y., Mejía-Muñoz, E., Polanco-Fierro, J. A., and García-Sánchez, J. R. (2021). A moderate intensity exercise program improves physical function and oxidative damage in older women with and without sarcopenic obesity. *Experimental Gerontology*, *150*, 111360.

Hailemariam, M. B., Woldu, Z., Asfaw, Z., and Lulekal, E. (2021). Ethnobotany of an indigenous tree *Piliostigma thonningii* (Schumach.) Milne-Redh(Fabaceae) in the arid and semi-arid areas of South Omo Zone, southern Ethiopia. *Journal of Ethnobiology and Ethnomedicine*, *17*(1), 1-18.

Halim, M. A., Kanan, K. A., Nahar, T., Rahman, M. J., Ahmed, K. S., Hossain, H., ... and Ahmed, M. (2022). Metabolic profiling of phenolics of the extracts from the various parts of blackberry plant (Syzygium cumini L.) and their antioxidant activities. *LWT*, *167*, 113813.

Harborne, A. J. (1998). *Phytochemical Methods a Guide to Modern Techniques of Plant Analysis*. springer science and business media.

Hossain, M. S., Islam, M. D., Galib, A., Malek, R., Akter, K., and Khanam, M. (2021). Lipid Profile in Relation to Body Mass Index among Medical College Students in Dhaka, Bangladesh. *Medicine Today*, *33*(2), 114-119.

Huang, J., Wang, Y., Xie, Z., Zhou, Y., Zhang, Y., and Wan, X. (2014). The anti-obesity effects of green tea in human intervention and basic molecular studies. *European Journal of Clinical Nutrition*, *68*(10), 1075-1087.

Ibrahim, H., Uttu, A. J., Sallau, M. S., and Iyun, O. R. A. (2021). Gas chromatography–mass spectrometry (GC–MS) analysis of ethyl acetate root bark extract of *Strychnos innocua* (Delile). *Beni-Suef University Journal of Basic and Applied Sciences*, *10*,1-8.

Iftikhar, N., Hussain, A. I., Chatha, S. A. S., Sultana, N., and Rathore, H. A. (2022). Effects of polyphenol-rich traditional herbal teas on obesity and oxidative stress in rats fed a high-fat–sugar diet. *Food Science and Nutrition*, *10*(3), 698-711.

Islam, S. U., Ahmed, M. B., Ahsan, H., and Lee, Y. S. (2021). Recent molecular mechanisms and beneficial effects of phytochemicals and plant-based whole foods in reducing LDL-C and preventing cardiovascular disease. *Antioxidants*, *10*(5), 784.

Jaca, A., Iwu, C., Durão, S., Onyango, A. W., and Wiysonge, C. S. (2020). Understanding the underlying drivers of obesity in Africa: a scoping review protocol. *BMJ open*, *10*(11), e040940.

Jamshidi-Kia, F., Lorigooini, Z., and Amini-Khoei, H. (2017). Medicinal plants: Past history and future perspective. *Journal of Herbmed Pharmacology*, *7*(1), 1-7.

Jang, W. S., and Choung, S. Y. (2013). Antiobesity effects of the ethanol extract of *Laminaria japonica* Areshoung in high-fat-diet-induced obese rat. *Evidence-Based Complementary and Alternative Medicine*, *2013*.

Jaradat, N., Khasati, A., Hawi, M., Hawash, M., Shekfeh, S., Qneibi, M., ... and Qaoud, M. T. (2022). Antidiabetic, antioxidant, and anti-obesity effects of phenylthio-ethyl benzoate derivatives, and molecular docking study regarding α-amylase enzyme. *Scientific Reports*, *12*(1), 3108.

Johnson, S., and Schwartz, S. M. (2018). Pharmacologic and pharmacodynamic equivalence of 2 formulations of orlistat. *Clinical Pharmacology in Drug Development*, *7*(7), 773-780.

Kansra, A. R., Lakkunarajah, S., and Jay, M. S. (2021). Childhood and adolescent obesity: A review. *Frontiers in Pediatrics*, *8*, 581461.

Kareru, P. G., Gachanja, A. N., Keriko, J. M., and Kenji, G. M. (2008). Antimicrobial activity of some medicinal plants used by herbalists in eastern province, Kenya. *African Journal of Traditional, Complementary and Alternative Medicines*, *5*(1), 51-55.

Kaur, R., Kaur, J., Mahajan, J., Kumar, R., and Arora, S. (2014). Oxidative stress—implications, source and its prevention. *Environmental Science and Pollution Research*, *21*, 1599-1613.

Kelleni, M. T. (2018). Role of central and peripheral serotonin in obesity: what to expect in the near pharmacotherapy future. *Advanced Obesity Weight Managment Control*, *8*(6), 291.

Kennedy, D. O., and Wightman, E. L. (2011). Herbal extracts and phytochemicals: plant secondary metabolites and the enhancement of human brain function. *Advances in Nutrition*, *2*(1), 32-50.

Kim, E., Cui, J., Zhang, G., and Lee, Y. (2022). Physiological effects of green-colored food-derived bioactive compounds on cardiovascular and metabolic diseases. *Applied Sciences*, *12*(4), 1879.

Kim, G. W., Lin, J. E., Blomain, E. S., and Waldman, S. A. (2014). Antiobesity pharmacotherapy: new drugs and emerging targets. *Clinical Pharmacology and Therapeutics*, *95*(1), 53-66.

Kim, S. Y., Oh, M. R., Kim, M. G., Chae, H. J., and Chae, S. W. (2015). Anti-obesity effects of *Yerba Mate* (*Ilex Paraguariensis*): a randomized, double-blind, placebo-controlled clinical trial. *BMC complementary and alternative medicine*, *15*(1), 1-8.

Kimathi, P. K., Maitho, T., Mbaria, J., and Moriasi, G. (2022). Antidiarrheal, antimicrobial, and toxic effects of the aqueous and methanolic leaf and fruit extracts of *Cucumis dipsaceus* (Ehrenb. Ex Spach.). *Journal of Herbmed Pharmacology*, *11*(2), 213-225.

Klaver, M., De Blok, C. J. M., Wiepjes, C. M., Nota, N. M., Dekker, M. J., de Mutsert, R., and Den Heijer, M. (2018). Changes in regional body fat, lean body mass and body shape in trans persons using cross-sex hormonal therapy: results from a multicenter prospective study. *European Journal of Endocrinology*, *178*(2), 163-171.

Klop, B., Elte, J. W. F., and Castro Cabezas, M. (2013). Dyslipidemia in obesity: mechanisms and potential targets. *Nutrients*, *5*(4), 1218-1240.

Koche, D., Shirsat, R., and Kawale, M. A. H. E. S. H. (2016). An overerview of major classes of phytochemicals: their types and role in disease prevention. *Hislopia Journal*, *9*(1/2), 0976-2124.

Kokwaro, J. O. (2009). *Medicinal Plants of East Africa*. University of Nairobi press.

Kumar, V., Singh, D. D., Lakhawat, S. S., Yasmeen, N., Pandey, A., and Singla, R. K. (2022). Biogenic phytochemicals modulating obesity: From molecular mechanism to preventive and therapeutic approaches. *Evidence-Based Complementary and Alternative Medicine*, *2022*.

Kwaji, A., Bassi, P. U., Aoill, M., Nneji, C. M., and Ademowo, G. (2010). Preliminary studies on *Piliostigma thonningii* Schum leaf extract: Phytochemical screening and in vitro antimalarial activity. *African Journal of*

Microbiology Research, *4*(9), 735-739.

Lee, H. G., Lu, Y. A., Li, X., Hyun, J. M., Kim, H. S., Lee, J. J., and Jeon, Y. J. (2020). Anti-Obesity effects of *Grateloupia elliptica*, a red seaweed, in mice with high-fat diet-induced obesity via suppression of adipogenic factors in white adipose tissue and increased thermogenic factors in brown adipose tissue. *Nutrients*, *12*(2), 308.

Lee, J. Y., Seo, K. H., Lee, E. Y., Ji, Y. J., Lee, Y. J., Kang, M. H., and Kim, H. D. (2021). Antioxidant and Anti-Obesity Potentials of Korean-Native Wild Vegetables (Allium species). *Horticulturae*, *7*(12), 541.

Lee, M. O. (1929). Determination of the surface area of the white rat with its application to the expression of metabolic results. *American Journal of Physiology-Legacy Content*, *89*(1), 24-33.

Lee, Y. H., Mottillo, E. P., and Granneman, J. G. (2014). Adipose tissue plasticity from WAT to BAT and in between. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, *1842*(3), 358-369.

Leopoldo, A. S., Lima-Leopoldo, A. P., Nascimento, A. F., Luvizotto, R. A. M., Sugizaki, M. M., Campos, D. H. S., and Cicogna, A. C. (2016). Classification of different degrees of adiposity in sedentary rats. *Brazilian Journal of Medical and Biological Research*, *49*.

Leung, W. Y., Thomas, G. N., Chan, J. C., and Tomlinson, B. (2003). Weight management and current options in pharmacotherapy: orlistat and sibutramine. *Clinical Therapeutics*, *25*(1), 58-80.

Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., and Abete, P. (2018). Oxidative stress, aging, and diseases. *Clinical interventions in aging*, 757-772.

Lin, X., and Li, H. (2021). Obesity: Epidemiology, Pathophysiology, and Therapeutics. In Frontiers in Endocrinology (Vol. 12). Frontiers Media S.A.

Liu, H., and Lu, H. Y. (2014). Nonalcoholic fatty liver disease and cardiovascular disease. *World Journal of Gastroenterology: WJG*, *20*(26), 8407.

Liu, Y., Sun, M., Yao, H., Liu, Y., and Gao, R. (2017). Herbal medicine for the treatment of obesity: an overview of scientific evidence from 2007 to 2017. *Evidence-Based Complementary and Alternative Medicine*, *2017*.

Lustig, R. H., Collier, D., Kassotis, C., Roepke, T. A., Kim, M. J., Blanc, E., and Heindel, J. J. (2022). Obesity I: Overview and molecular and biochemical mechanisms. *Biochemical Pharmacology*, 115012.

Ma, M., Liu, X., Jia, G., Geng, B., and Xia, Y. (2022). The association between body fat distribution and bone mineral density: evidence from the US population. *BMC Endocrine Disorders*, *22*(1), 170.

Makosa, M., Sithole, S., and Mukanganyama, S. (2021). Evaluation of the antimicrobial effect of the extracts of the pods of *Piliostigma thonningii* (schumach.) milne-redh (Fabaceae). *Advances in Pharmacological and Pharmaceutical Sciences*, *2021*.

Manguro, L. O. A., Owuor, P. O., and Ochung, A. A. (2018). Isolation, characterization and biological activities of phytoconstituents from *Lonchocarpus eriocalyx* Harms leaves.

Marseglia, L., Manti, S., D'Angelo, G., Nicotera, A., Parisi, E., Di Rosa, G., and Arrigo, T. (2014). Oxidative stress in obesity: a critical component in human diseases. *International Journal of Molecular Sciences*, *16*(1), 378-400.

Mohamed, S. F., Haregu, T. N., Khayeka-Wandabwa, C., Muthuri, S. K., and Kyobutungi, C. (2019). Magnitude and predictors of normal-weight central

obesity–the AWI-Gen study findings. *Global Health Action*, *12*(1), 1685809.

Morgan, A., Mooney, K., and Mc Auley, M. (2016). Obesity and the dysregulation of fatty acid metabolism: implications for healthy aging. *Expert review of endocrinology and metabolism*, *11*(6), 501-510.

Moriasi, G. A., Ireri, A. M., and Ngugi, M. P. (2020). In vivo cognitive-enhancing, ex vivo malondialdehyde-lowering activities and phytochemical profiles of aqueous and methanolic stem bark extracts of *Piliostigma thonningii* (schum.). *International Journal of Alzheimer's Disease*, *2020*.

Moriasi, G. A., Ireri, A. M., Nelson, E. M., and Ngugi, M. P. (2021). In vivo anti-inflammatory, anti-nociceptive, and in vitro antioxidant efficacy, and acute oral toxicity effects of the aqueous and methanolic stem bark extracts of *Lonchocarpus eriocalyx* (Harms.). *Heliyon*, *7*(5), e07145.

Moriasi, G. A., Kibiti, C. M., and Ngugi, M. P. (2021). In vivo antidiabetic efficacy, mineral element composition, and qualitative phytochemistry of the aqueous leaf extracts of *Pentas zanzibarica*

Moriasi, G., Ireri, A., and Ngugi, M. P. (2020). In vitro antioxidant activities of the aqueous and methanolic stem bark extracts of *Piliostigma thonningii* (Schum.). *Journal of Evidence-Based Integrative Medicine*, *25*,

Moriasi, G., Nelson, E., and Twahirwa, E. (2020). In vitro anti-inflammatory, antioxidant and qualitative phytochemical evaluation of the Phytexponent preparation of selected plants.

Mubiu, J. K., Ndwigah, S. N., Abuga, K. O., and Ongarora, D. S. (2017). Antimicrobial activity of extracts and phytosterols from the root bark of *Lonchocarpus eriocalyx*. *East and Central African Journal of Pharmaceutical Sciences*, *20*(1-3), 13-16.

Nedergaard, J., and Cannon, B. (2017). Brown Adipose Tissue. In Fetal and Neonatal Physiology

Nderitu, K. W., Mwenda, N. S., Macharia, N. J., Barasa, S. S., and Ngugi, M. P. (2017). Antiobesity activities of methanolic extracts of *Amaranthus dubius, Cucurbita pepo,* and *Vigna unguiculata* in progesterone-induced obese Mice. *Evidence-Based Complementary and Alternative Medicine*, *2017*.

Nicolucci, A., and Maffeis, C. (2022). The adolescent with obesity: what perspectives for treatment. *Italian Journal of Pediatrics*, *48*(1), 1-9.

Nittari, G., Khuman, R., Baldoni, S., Pallotta, G., Battineni, G., Sirignano, A., and Ricci, G. (2020). Telemedicine practice: review of the current ethical and legal challenges. *Telemedicine and e-Health*, *26*(12), 1427-1437.

Nonogaki, K. (2022). The regulatory role of the central and peripheral serotonin network on feeding signals in metabolic diseases. *International Journal of Molecular Sciences*, *23*(3), 1600.

Noufou, O., Andre, T., Richard, S. W., Yerbanga, S., Maminata, T., Ouedraogo, S., and Pierre, G. I. (2016). Anti-inflammatory and anti-plasmodial activities of methanol extract of *Pterocarpus erinaceus* Poir(Fabaceae) leaves. *International Journal of Pharmacology*, *12*(5), 549-555.

Nwobodo, N. N. (2015). Toxicity and safety concerns in orlistat therapy for obesity: A critical evaluation. *Asian Journal of Biomedical and Pharmaceutical Sciences*, *5*(47), 1.

OCHUNG, A. A. (2016). Phytochemical investigations of *Lonchocarpus eriocalyx* (Harms), *Alysicarpus ovalifolius* (Schumach) and *Erythrina abyssinica* (DC) for antiplasmodial, larvicidal, mosquitocidal and antimicrobial activities (Doctoral dissertation, Maseno University).

Olela, B. O. (2021). Acute Oral-toxicity, Anti-inflammatory and Analgesic Effects of Aqueous and Methanolic Bark Extracts of *Piliostigma Thonningii (Schum)* in Mice (Doctoral dissertation, University of Nairobi).

Olela, B., Mbaria, J., Wachira, T., and Moriasi, G. (2020). Acute oral toxicity and anti-inflammatory and analgesic effects of aqueous and methanolic stem bark extracts of *Piliostigma thonningii* (Schumach.). *Evidence-Based Complementary and Alternative Medicine*, *2020*.

Olela, B., Mbaria, J., Wachira, T., and Moriasi, G. (2020). Acute oral toxicity and anti-inflammatory and analgesic effects of aqueous and methanolic stem bark extracts of *Piliostigma thonningii* (Schumach.). *Evidence-Based Complementary and Alternative Medicine*, *2020*.

Orwa C., Mutua A., R, Kindt., R., J., and S.., A. (2009). *Piliostigma thonningii* (Schum) Milne-Redh. Aggroforestry Database 4.0, 0, 1–5.

Onyango, E. M., and Onyango, B. M. (2018). The rise of non-communicable diseases in Kenya: an examination of the time trends and contribution of the changes in diet and physical inactivity. *Journal of Epidemiology and Global Health*, *8*(1-2), 1.

Ozcan, A., and Ogun, M. (2015). Biochemistry of reactive oxygen and nitrogen species. *Basic Principles and Clinical Significance of Oxidative Stress*, *3*, 37-58.

Pan, M. H., Wu, J. C., Ho, C. T., and Lai, C. S. (2018). Anti-obesity molecular mechanisms of action: Resveratrol and pterostilbene. *Biofactors*, *44*(1), 50-60.

Park, S. H., Huh, T. L., Kim, S. Y., Oh, M. R., Tirupathi Pichiah, P. B., Chae, S. W., and Cha, Y. S. (2014). Anti-obesity effect of *Gynostemma pentaphyllum* extract (actiponin): a randomized, double-blind, placebo-controlled trial. *Obesity*, *22*(1), 63-71.

Payab, M., Hasani-Ranjbar, S., Aletaha, A., Ghasemi, N., Qorbani, M., Atlasi, R., and Larijani, B. (2018). Efficacy, safety, and mechanisms of herbal medicines used in the treatment of obesity: A protocol for systematic review. *Medicine*, *97*(1).

Pérez-Torres, I., Castrejón-Téllez, V., Soto, M. E., Rubio-Ruiz, M. E., Manzano-Pech, L., and Guarner-Lans, V. (2021). Oxidative stress, plant natural antioxidants, and obesity. *International Journal of Molecular Sciences*, *22*(4), 1786.

Pérez-Torres, I., Castrejón-Téllez, V., Soto, M. E., Rubio-Ruiz, M. E., Manzano-Pech, L., and Guarner-Lans, V. (2021). Oxidative stress, plant natural antioxidants, and obesity. *International Journal of Molecular Sciences*, *22*(4), 1786.

Piero, N. M., Eliud, N. N. M., Susan, K. N., George, O. O., and David, N. J. M. M. (2015). In vivo antidiabetic activity and safety in rats of *Cissampelos pareira* traditionally used in the management of diabetes mellitus in Embu County, Kenya.

Piqueras, P., Ballester, A., Durá-Gil, J. V., Martinez-Hervas, S., Redón, J., and Real, J. T. (2021). Anthropometric indicators as a tool for diagnosis of obesity and other health risk factors: a literature review. *Frontiers in Psychology*, *12*, 631179.

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., ... and Bitto, A. (2017). Oxidative stress: harms and benefits for human health. *Oxidative Medicine and Cellular Longevity*, *2017*.

Purnell, J. Q. (2018). Definitions, classification, and epidemiology of

obesity. *Endotext [Internet]*.

Qi, X. (2018). Review of the clinical effect of orlistat. In *IOP Conference Series: Materials Science and Engineering* (Vol. 301, No. 1, p. 012063). IOP Publishing.

Rana, K., Ghimire, P., Chimoriya, R., and Chimoriya, R. (2021). Trends in the prevalence of overweight and obesity and associated socioeconomic and household environmental factors among women in Nepal: Findings from the Nepal demographic and health surveys. *Obesities*, *1*(2), 113-135.

Rivera-Ramírez, F., Escalona-Cardoso, G. N., Garduno-Siciliano, L., Galaviz-Hernández, C., and Paniagua-Castro, N. (2011). Antiobesity and hypoglycaemic effects of aqueous extract of *Ibervillea sonorae* in mice fed a high-fat diet with fructose. *Journal of Biomedicine and Biotechnology*, *2011*.

Rodgers, R. J., Tschöp, M. H., and Wilding, J. P. (2012). Anti-obesity drugs: past, present and future. *Disease Models and Mechanisms*, *5*(5), 621-626.

Rodríguez-Pérez, C., Segura-Carretero, A., and del Mar Contreras, M. (2019). Phenolic compounds as natural and multifunctional anti-obesity agents: A review. *Critical Reviews in Food Science and Nutrition*, *59*(8), 1212-1229.

Rome, E. S. (2011). Obesity prevention and treatment. *Pediatrics in Review-Elk Grove*, *32*(9), 363.

Saad, B., Ghareeb, B., and Kmail, A. (2021). Metabolic and epigenetics action mechanisms of antiobesity medicinal plants and phytochemicals. *Evidence-Based Complementary and Alternative Medicine*, *2021*, 1-19.

Salmerón-Manzano, E., Garrido-Cardenas, J. A., and Manzano-Agugliaro, F. (2020). Worldwide research trends on medicinal plants. *International Journal of Environmental Research and Public Health*, *17*(10), 3376.

Santos, H. O., and Lavie, C. J. (2021). Weight loss and its influence on high-density lipoprotein cholesterol (HDL-C) concentrations: A noble clinical hesitation. *Clinical Nutrition ESPEN*, *42*, 90-92.

Seidell, J. C., and Halberstadt, J. (2015). The global burden of obesity and the challenges of prevention. *Annals of Nutrition and Metabolism*, *66*(Suppl. 2), 7-12.

Shahid, S. U., and Sarwar, S. (2020). The abnormal lipid profile in obesity and coronary heart disease (CHD) in Pakistani subjects. *Lipids in Health and Disease*, *19*(1), 1-7.

Shalitin, S., and Gat-Yablonski, G. (2022). Associations of obesity with linear growth and puberty. *Hormone Research in Paediatrics*, *95*(2), 120-136. DOI: 10.1159/000516171

Sharifi-Rad, M., Anil Kumar, N. V., Zucca, P., Varoni, E. M., Dini, L., Panzarini, E., and Sharifi-Rad, J. (2020). Lifestyle, oxidative stress, and antioxidants: back and forth in the pathophysiology of chronic diseases. *Frontiers in physiology*, *11*, 694.

Sigei, E. C., Okinyi, V. P., Kariuki, P. K., Kariuki, L., and Kimani, J. (2018). Prevalence of Overweight/Obesity and Some Associated Factors among Rural/Urbanites of Uasin-Gishu County, Kenya: A Cross-Sectional Study. *Journal of Nutritional Sciences and Dietetics*, 109-116.

Sims, J., Lutz, E., Wallace, K., Kassahun-Yimer, W., Ngwudike, C., and Shwayder, J. (2020). Depo-medroxyprogesterone acetate, weight gain and amenorrhea among obese adolescent and adult women. *The European Journal of Contraception and Reproductive Health Care*, *25*(1), 54-59.

Singh, O., Gupta, M., and Khajuria, V. (2015). Lipid profile and its relationship

with blood glucose levels in metabolic syndrome. *National Journal of Physiology, Pharmacy and Pharmacology*, *5*(2), 134.

Sombra, L. R. S., and Anastasopoulou, C. (2022). Pharmacologic therapy for obesity. In *StatPearls [Internet]*. StatPearls Publishing.

Tang, H., Xiang, Z., Li, L., Shao, X., Zhou, Q., You, X., and Zou, H. (2021). Potential role of anti-inflammatory HDL subclasses in metabolic unhealthy/obesity. *Artificial Cells, Nanomedicine, and Biotechnology*, *49*(1), 564-574.

Taylor, B. A., and Phillips, S. J. (1996). Detection of obesity QTLs on mouse chromosomes 1 and 7 by selective DNA pooling. *Genomics*, *34*(3), 389-398.

Tchang, B. G., Saunders, K. H., and Igel, L. I. (2021). Best practices in the management of overweight and obesity. *Medical Clinics*, *105*(1), 149-174.

Thomas, S. S., Kim, M., Lee, S. J., and Cha, Y. S. (2018). Antiobesity effects of purple perilla (*Perilla frutescens* var. acuta) on adipocyte differentiation and mice fed a high-fat diet. *Journal of Food Science*, *83*(9), 2384-2393.

Truong, D. H., Nguyen, D. H., Ta, N. T. A., Bui, A. V., Do, T. H., and Nguyen, H. C. (2019). Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of *Severinia buxifolia*. *Journal of Food Quality*, *2019*.

Vekic, J., Zeljkovic, A., Stefanovic, A., Jelic-Ivanovic, Z., and Spasojevic-Kalimanovska, V. (2019). Obesity and dyslipidemia. *Metabolism*, *92*, 71-81.

Verdile, G., Keane, K. N., Cruzat, V. F., Medic, S., Sabale, M., Rowles, J., and Newsholme, P. (2015). Inflammation and oxidative stress: the molecular connectivity between insulin resistance, obesity, and Alzheimer's disease. *Mediators of Inflammation*, *2015*.

Wang, S., Ji, X., Zhang, Z., and Xue, F. (2020). Relationship between lipid profiles and glycemic control among patients with type 2 diabetes in Qingdao, China. *International Journal of Environmental Research and Public Health*, *17*(15), 5317.

WHO. (2022). Obesity rising in Africa, WHO analysis finds | WHO | Regional Office for Africa. Report. https://www.afro.who.int/news/obesity-rising-africa-who-analysis-finds.

Wiechert, M., and Holzapfel, C. (2021). Nutrition Concepts for the Treatment of Obesity in Adults. Nutrients 2022, 14, 169. *Recent Advances and Remaining Challenges*, 1.

Willey, J. Z., Gardener, H., Moon, Y. P., Yoshita, M., DeCarli, C., Cheung, Y. K., ... and Wright, C. B. (2014). Lipid profile components and subclinical cerebrovascular disease in the northern Manhattan study. *Cerebrovascular Diseases*, *37*(6), 423-430.

Xia, J. H., Zhang, D. Y., Kang, Y. Y., Guo, Q. H., Cheng, Y. B., Huang, J. F., and Wang, J. G. (2022). The prevalence of masked hypertension and masked uncontrolled hypertension in relation to overweight and obesity in a nationwide registry in China. *Hypertension Research*, *45*(11), 1690-1700.

Yang, C., Li, L., Yang, L., Lǚ, H., Wang, S., and Sun, G. (2018). Anti-obesity and Hypolipidemic effects of garlic oil and onion oil in rats fed a high-fat diet. *Nutrition and Metabolism*, *15*(1), 1-8.

Yeh, J. H., Tung, Y. T., Yeh, Y. S., and Chien, Y. W. (2021). Effects of dietary fatty acid composition on lipid metabolism and body fat accumulation in ovariectomized rats. *Nutrients*, *13*(6), 2022.

Yen, G. C., Cheng, H. L., Lin, L. Y., Chen, S. C., and Hsu, C. L. (2020). The

potential role of phenolic compounds on modulating gut microbiota in obesity. *Journal of Food and Drug Analysis*, *28*(2), 195.

Yusefzadeh, H., Rashidi, A., and Rahimi, B. (2019). Economic burden of obesity: A systematic review. *Social Health and Behavior*, *2*(1), 7.

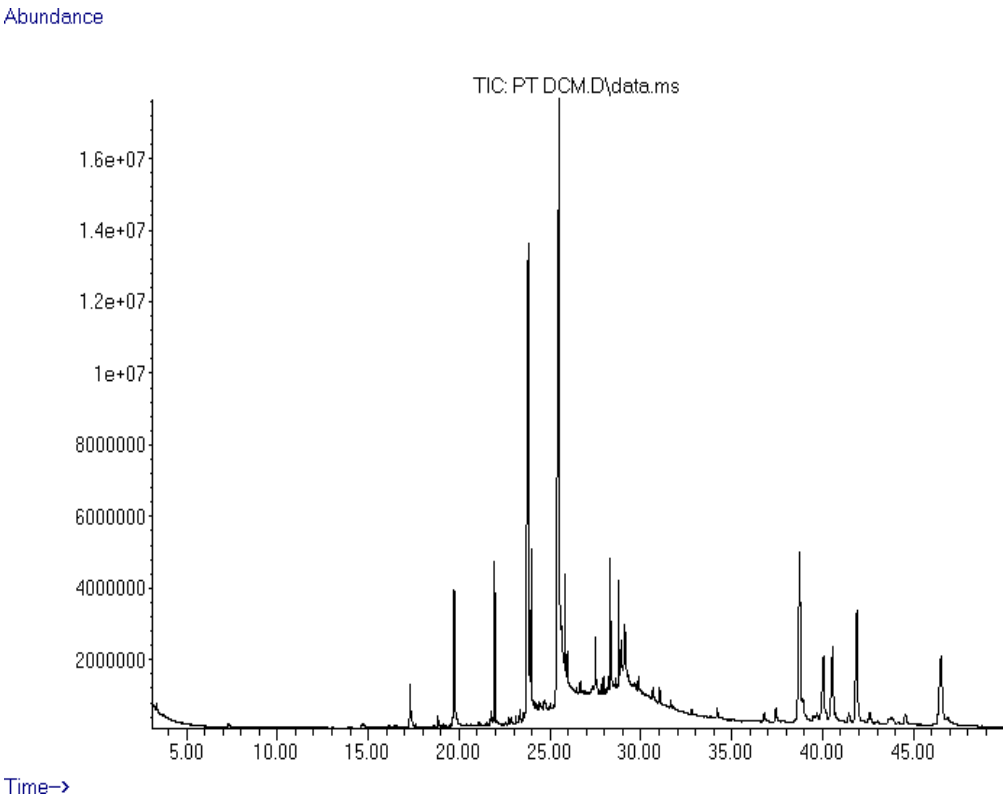
Zalejska-Fiolka, J., Hubková, B., Birková, A., Veliká, B., Puchalska, B., Kasperczyk, S., and Birkner, E. (2019). Prognostic value of the modified atherogenic index of plasma during body mass reduction in Polish obese/overweight people. *International Journal of Environmental Research and Public Health*, *16*(1), 68.

Zaurov, D. E., Belolipov, I. V., Kurmukov, A. G., Sodombekov, I. S., Akimaliev, A. A., and Eisenman, S. W. (2013). The medicinal plants of Uzbekistan and Kyrgyzstan. *Medicinal Plants of Central Asia: Uzbekistan and Kyrgyzstan*, 15-273.

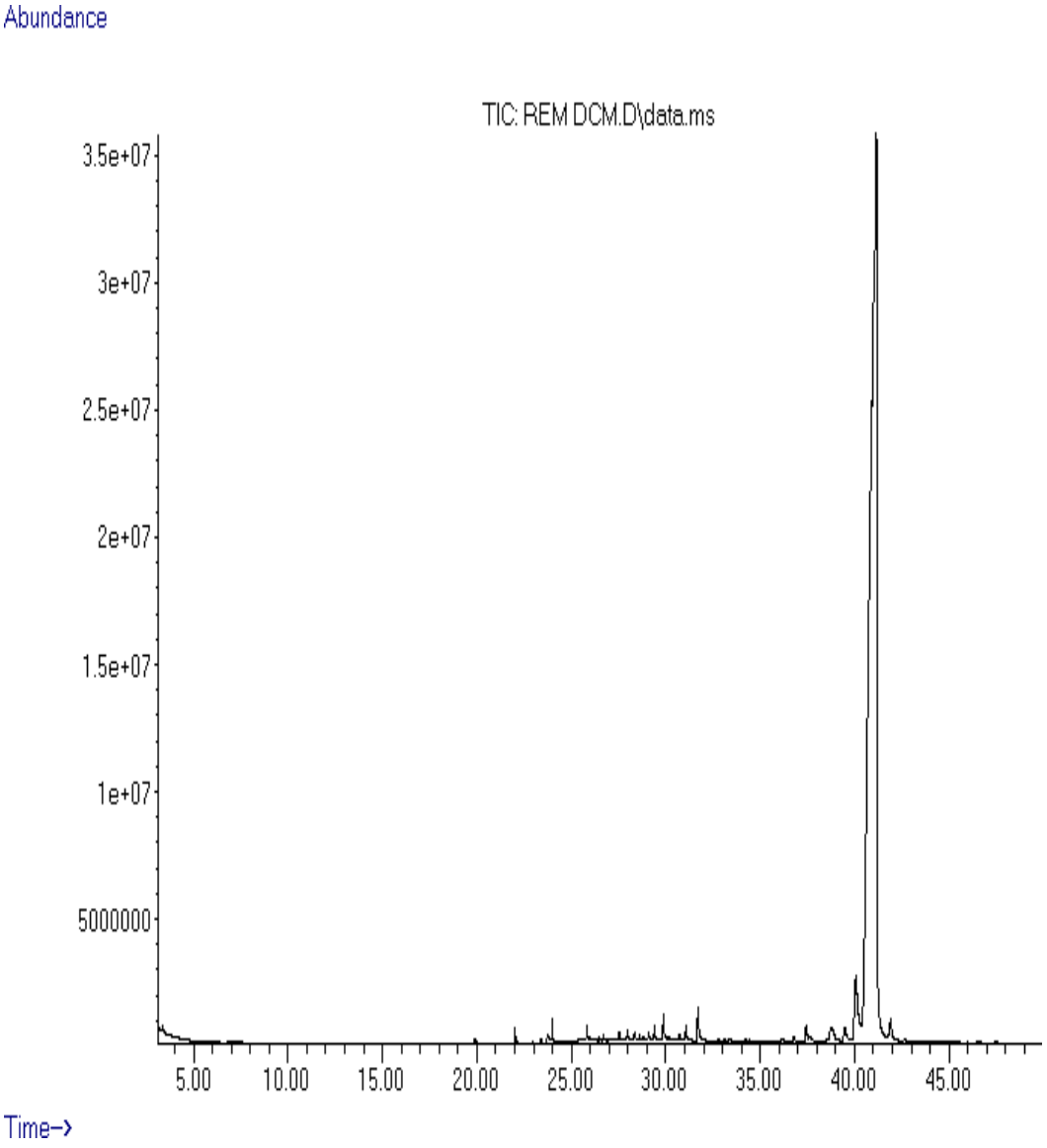
Zielinska-Blizniewska, H., Sitarek, P., Merecz-Sadowska, A., Malinowska, K., Zajdel, K., Jablonska, M., and Zajdel, R. (2019). Plant extracts and reactive oxygen species as two counteracting agents with anti-and pro-obesity properties. *International Journal of Molecular Sciences*, *20*(18), 4556.

APPENDICES

Appendix I: GC-MS chromatogram for *P. thonningii* stem bark DCM extracts with retention time



Appendix II: GC-MS chromatogram for *L. eriocalyx* stem bark DCM extracts with retention time



Appendix III: National Commission for Science, Technology, and Innovation permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

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Date of Issue: **15/December/2022**

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