HIGH DOSE YOYO CLEANSER BITTERS: EVALUATING PREVENTIVE POTENTIALS AMID CHANGES IN LIPID PROFILE

BY

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CERTIFICATION

We the undersigned hereby certify that Mr. Olanrewaju Timothy Awosika carried out this work, in the Department of Medical Biochemistry, University of Benin, Benin City and we approve same as adequate in scope and quality for the award of Bachelor of Science Degree (B.Sc.) in Medical Biochemistry.

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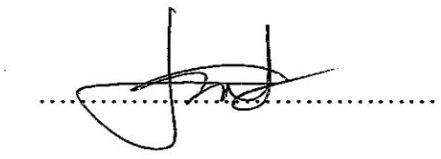

External Examiner

06/06/24

Date

DEDICATION

“In every step of this journey, I acknowledge and give thanks to God, for ‘I can do all things through Christ who strengthens me’ (Phillipians 4:13). To Him, I dedicate this project. I’m grateful for his unwavering grace and guidance that sustained me throughout this academic pursuit.

I also extend heartfelt appreciation to my family for their boundless love, encouragement, and sacrifices. Your support has been the bedrock of my achievements.

Lastly, I dedicate this work to myself, a testament of preserverance and determination amidst challenges.

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ABSTRACT

This study evaluated the effects of Yoyo cleanser bitters on male *Wistar* rats fed with a high fructose diet. Yoyo Cleanser Bitters, a traditional herbal preparation, is purported to possess potential therapeutic properties for metabolic disorders. Twenty (20) male *Wistar* rats weighing an average of 220grams each were randomly divided in four groups of five. The rats were fed *ad-libitum* with the feed and clean tap water during the entire course of the experiment (56 days). A basal diet of a standard pelleted grower's marsh was given to the control group (group1), while the treatment diet of 60% high fructose diet along with 20% fructose water was fed to the other three cages. Atorvastatin (0.57mg/kg) was administered daily to group 3 and Yoyo cleanser bitters (1200 mg/kg) was administered to the rats in group 4. The treatment were administered using an oro-gastric gavage. Using standard methods, the weekly weight of the rats, their daily food consumption, feed efficiency were determined at the end of the 56days of study. Comprehensive biochemical assessment of lipid profile was performed. The data was analyzed using the one-way analysis of variance (ANOVA), followed by Tukey's test of significance. A p value of less than 0.05 ($p<0.05$) was accepted as statistically significant ($p<0.05$). The results of the study revealed that High Yoyo cleanser bitters posses preventive properties related to metabolic disorders in male *Wistar* rats when compared with both control and Atorvastatin groups as it significantly reduced ($p<0.05$) the levels of Triglycerides, Low density lipoprotein, Very Low-density Lipoprotein levels yet increasing the high density lipoprotein levels. This is an indication that the mechanism of action of Yoyo Cleanser Bitters can be added as a preventive intervention for individuals at the risk of dyslipidemia.

CHAPTER ONE

INTRODUCTION

1.0 Background of Study

Fructose, a widely present monosaccharide in the human diet, plays a vital role in essential metabolic processes. It is found in honey, fruits, and vegetables, and is a constituent of high-fructose corn syrup (HFCS), extensively utilized in the production of soft drinks and various food items. The consumption of these products significantly contributes to the intake of added sugars in the diet, with approximately half of them being in the form of fructose. In the stomach, sucrose (table sugar) undergoes acid hydrolysis, transforming into fructose and glucose, followed by further breakdown in the small intestine through sucrase-isomaltase action (Sloboda *et al.,* 2014). Also recognized as fruit sugar, this monosaccharide is twice as sweet as glucose and traditionally consumed in a balanced fructose-to-glucose ratio, along with the accompanying fiber, vitamins, and minerals found in fruits. However, Western diets now incorporate increasing amounts of free fructose, with certain soft drinks containing twice as much fructose compared to glucose (Walker *et al.,* 2014). The inclusion of fructose in various food items dates back to 1957 when a commercial method to convert glucose into fructose was developed, facilitating the production of high-fructose corn syrup (HFCS), which contains both glucose and fructose as monosaccharides (Douard and Ferraris, 2008). The relatively low production cost of HFCS has contributed to the escalating intake of fructose through processed foods and beverages. There is significant evidence indicating that an overabundance of fructose in the diet has adverse impacts on various metabolic disorders. It results in the buildup of visceral fat, contributing to conditions such as obesity, hyperlipidemia, insulin

resistance, hypertension, and hyperuricemia (Malik *et al.,* 2019). These factors are closely linked to the onset of diseases such as diabetes, fatty liver disease, cardiovascular issues, and gout (Xiaofeng *et al.,* 2022). Even among individuals with fructose consumption within the "normal" range, fructose can swiftly compromise essential physiological markers such as circulating lipids and insulin sensitivity in humans (Stanhope *et al.,* 2015). Additionally, heightened fructose intake has been linked to impaired signaling of appetite hormones and compromised neuronal health (Lindqvist *et al.,* 2008).

A lipid profile refers to the composition of lipids in the bloodstream, encompassing total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL). Typically, this set of tests is conducted collectively to assess the risk of heart disease, serving as potential indicators of the likelihood of a heart attack or stroke due to blood vessel blockage or arterial hardening. For an individual weighing around 68 kg, the average daily synthesis of total blood cholesterol is approximately 1 g (1000 mg). Elevated levels of TC, TG, and LDL are associated with an increased risk of coronary artery disease (CAD) and ischemic stroke (Natarajan *et al.,* 2010). Conversely, population-based studies consistently reveal an inverse association between HDL levels and CAD risk (Adak *et al.,* 2010). Additionally, patients with metabolic syndrome (MetS), a population at high risk of developing cardiovascular disease (CVD), exhibit various lipid abnormalities beyond elevated LDL-c, including heightened triglyceride levels, low HDL cholesterol (HDL-c), and increased small and dense LDL (sd-LDL) particles (Ruotolo and Howard 2002).

YoYo Bitters is a popular herbal oral remedy widely utilized in Southwest Nigeria. Comprising a combination of plant components like Aloe vera, Cinnamum aromaticum, Citrus aurantifolia, Acinos arvensis, and Chenopodium murale, this preparation is reputed for its efficacy in preventing kidney and bladder infections. Its asserted benefits extend to normalizing intestinal movement, regulating blood pressure, aiding digestion, and preventing conditions such as ulcers, gastritis, insomnia, stress, depression, and assisting in weight management (Kumdi *et al.,* 2011).

1.1 Justification of Study

The study on "High Dose Yoyo Cleanser Bitters: Evaluating Preventive Potential amid Changes in Lipid Profile" is essential because it investigates how taking larger amounts of Yoyo Cleanser Bitters can help protect against the harmful effects caused by eating too much fructose. When we consume too much fructose, it can lead to various health problems like gaining too much weight, having high cholesterol and blood sugar, and even developing diseases like diabetes and heart issues.

The study aims to find out if a high dose of Yoyo Cleanser Bitters can act like a shield, preventing these health problems from happening or reducing their severity. By focusing on how Yoyo Cleanser Bitters influences the levels of fats in our bodies, known as lipid profiles, the research aims to provide practical insights into whether this natural remedy can be a helpful and preventive solution for the health issues caused by a diet high in fructose.

This study matters because it addresses real health concerns that many people face due to their diets. If Yoyo Cleanser Bitters proves to be effective in preventing or lessening the impact of a high fructose diet on our health, it could offer a simple and natural way for people to take care of their well-being and make better choices in their daily lives.

1.2 Aim of Study

To determine the preventive potentials of high dose Yoyo cleanser bitters amid changes in lipid profile in male *Wistar* rats fed a high fructose diet.

1.3 Objectives of Study

1. To induce dyslipidemia in male *Wistar* rats fed with a high fructose diet.
2. To determine the effect of High dose Yoyo Cleanser Bitters on changes in serum levels of lipid profile parameters including total cholesterol, triglyceride, high-density lipoproteins and low-density lipoproteins, in rats fed high fructose diet.
3. To compare the effect of Atorvastatin to the potential therapeutic application of Yoyo Cleanser Bitters in alleviating dyslipidemia linked to the consumption of high fructose diet.

CHAPTER TWO

LITERATURE REVIEW

2.0 Fructose

Fructose, a ketonic monosaccharide, has the unique ability to be directly absorbed into the bloodstream from the gastrointestinal tract. Alongside glucose and galactose, it is one of three dietary monosaccharides with this property. Commonly sourced from sugar beets, sugar cane, and maize, fructose stands out as the sweetest among all monosaccharides (Benardout *et al.,* 2022). The food industry has harnessed the solubility and sweetness of fructose, especially in the production of artificial sweeteners. High fructose corn syrup (HFCS), a blend of glucose and fructose in the monosaccharide form, has gained significant popularity in recent years (Benardout *et al.,* 2022).

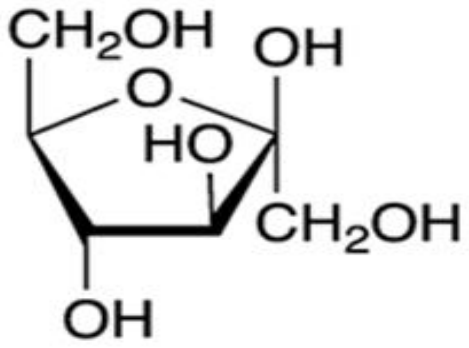

Fig 2.1: Fructose Structure (American Chemical Society, 2017).

The liver plays a crucial role in processing fructose following its absorption into the bloodstream from the intestines. In the liver, fructose is utilized for the synthesis of glucose, fatty acids, or lactate. Significant dietary sources of fructose encompass table sugar (sucrose, with equal proportions of fructose and glucose), high fructose corn

syrup (HFCS, constituting approximately 42–55% of energy as fructose and the remaining as glucose), fruits, and honey. The comparable composition of sucrose and HFCS, both containing nearly equal amounts of fructose and glucose, implies minimal differences in their effects (Schaefer *et al.,* 2009).

The consumption of sucrose, HFCS, and various sugars is estimated to contribute to approximately 25% of the energy intake in the United States. This percentage may be higher in certain groups like children, teenagers, African Americans, and Hispanics, largely due to a rise in the consumption of sugary soft drinks. The significant inclusion of added sugar in the human diet became prominent with the introduction of modern food processing. Subsequently, there has been a consistent upward trend in sugar consumption, particularly in soft drinks and fast-food items, leading to an increase in both total fat and overall energy intake (Schaefer *et al.,* 2009).

Several sugar transporters facilitate the utilization of fructose as a substrate, with GLUT2 and GLUT5 considered the primary physiological carriers. GLUT2, a high-capacity transporter, demonstrates low avidity for both glucose (KM = 17 mM) and fructose (KM = 66 mM). Unlike the insulin-sensitive glucose transporter GLUT4, GLUT2 is insulin-independent. It is present in the intestinal epithelium, where it contributes to sugar absorption, as well as in the kidney, liver, beta cells, and other tissues, where it plays a role in sensing blood glucose concentration and facilitating glucose and fructose transport (Cura and Carruthers, 2012).

On the other hand, GLUT5 exhibits a high specificity for fructose, despite its wide distribution beyond what would be anticipated based on its high KM of 6–10 mM and the typically low post-prandial circulating concentrations of fructose. While primarily found in the intestine, where it participates in fructose absorption, GLUT5 is also present in the kidney, fat, sperm, testes, brain, and skeletal muscle. The precise role of

GLUT5 and fructose metabolism in extraintestinal tissues remains incompletely understood (Douard and Ferraris, 2013).

Efficient fructose absorption from the gut is not commonly observed when fructose is consumed as the sole monosaccharide. This inefficiency is attributed to the low activity of GLUT5 and GLUT2 toward fructose. However, absorption is optimized when fructose is ingested in a 1:1 ratio with glucose, even though there is no apparent synergy between fructose and glucose at either GLUT2 or GLUT5. In rats, evidence suggests that the brush-border disaccharidase-related transport system, responsible for cleaving sucrose and transporting resulting sugar monomers, can also transport fructose and glucose when they are present together. Inhibition of this protein abolishes the ability of glucose to enhance fructose absorption (Laughlin, 2014). The extent to which free fructose enters circulation via the disaccharidase-related transport system in a normal diet and whether the kinetics of free fructose plus glucose absorption differ from that of sucrose remain unclear.

Metabolism of dietary fructose primarily occurs in the liver, bypassing the glycolytic rate-limiting step and leading to the build-up of intermediates. This process results in an excess of acetyl coenzyme A in the mitochondria, triggering de novo lipogenesis and the synthesis of fatty acids that can be esterified to produce triglycerides . A diet rich in fructose intensifies this metabolic pathway, and when coupled with a sedentary lifestyle, it may contribute to the development of various metabolic dysfunctions, including obesity, insulin resistance, glucose intolerance, high blood pressure, hypertriglyceridemia, reduced high-density lipoprotein (HDL) levels, and atherosclerosis (dson Souza-Pereira *et al.,* 2024).

2.1 FRUCTOSE AND METABOLIC SYNDROME

Metabolic syndrome (MetS) is a cluster of metabolic disorders, including central obesity, hyperglycemia, arterial hypertension, hypertriglyceridemia and low HDL-cholesterol, which affects about a quarter of the world's population (Saklayen, 2018). Fructose has been linked to the development of metabolic syndrome (MetS). Perez-Pozo et al. observed the emergence of MetS traits in healthy overweight adult men with the introduction of daily fructose intake alongside their regular diet (Perez-Pozo *et al.,* 2010). In a cross-sectional study, the association between dietary fructose consumption and MetS components, such as hyperglycemia, central obesity, and hypertriglyceridemia, was underscored (Chan *et al.,* 2019). Consequently, the inclusion of additional fructose in the diet may be regarded as a potential risk factor not only for MetS but also for non-alcoholic fatty liver disease (NAFLD).

The consumption of fructose-containing soft drinks has been associated with insulin resistance and changes in blood glucose levels. Kimber et al. demonstrated that prolonged intake of sugary beverages containing fructose exacerbates insulin sensitivity and glucose tolerance issues (Stanhope, 2009). Aeberli et al. similarly found a decrease in liver insulin sensitivity following moderate fructose consumption (Aeberli *et al.,* 2013). However, conflicting results emerged from a 24-week randomized controlled trial (RCT) where a low-fructose diet did not alleviate insulin resistance but did show improvement in fasting blood glucose levels by the end of the study period (Domínguez-Coello, 2020). The mechanism behind the disruption of glucose metabolism may be linked to hepatic insulin resistance resulting from prolonged exposure to fructose. In the liver, fructose promotes de novo lipogenesis (DNL), triggers endoplasmic reticulum stress and inflammation, ultimately reducing insulin sensitivity in hepatocytes (Smith *et al.,* 2020).

Consumption of sugary beverages and added fructose is linked to dyslipidemia. A high-fructose diet for 7 days resulted in increased secretion of VLDL from the liver (Saito *et al.,* 2013). Simultaneous ingestion of fructose-containing drinks with a fat mixture led to elevated serum triglycerides, as observed by Saito et al. (Saito *et al.,* 2013). Fructose promotes dyslipidemia through mechanisms involving de novo lipogenesis (DNL) and increased expression of Apo CIII. DNL, driven by fructose through upregulation of SREBP1c and ChREBP, contributes to the elevation of serum triglycerides. Apo CIII also contributes to hypertriglyceridemia by facilitating triglyceride mobilization during VLDL assembly and secretion. Additionally, Apo CIII impairs the cholesterol efflux capacity of HDL-c particles, exacerbating the dyslipidemia (Hieronimus *et al.,* 2020).

Obese individuals were found to have higher levels of added fructose intake (Chan *et al.,* 2019). Numerous studies have established a link between the consumption of soft drinks and weight gain (Miller *et al.,* 2020). Conversely, an intervention study involving a low added fructose diet demonstrated an improvement in central obesity (Domínguez-Coello *et al.,* 2020). The mechanisms underlying this effect are unrelated to caloric surplus and involve the regulation of insulin, leptin, and ghrelin. Fructose intake results in minimal insulin secretion, insufficient to stimulate the production of leptin by adipocytes. The absence of leptin synthesis hinders the initiation of satiety mechanisms, promoting further food intake (Crujeiras *et al.,* 2015). Additionally, fructose intake may contribute to the absence of ghrelin secretion inhibition, caused by reduced insulin secretion and glycemic stimulation following fructose ingestion. Lastly, uncontrolled fructose phosphorylation leads to ATP depletion in the liver. This ATP deficit, in turn, encourages additional energy intake through the diet, fostering weight gain (Bawden *et al.,* 2016).

2.2 HIGH FRUCTOSE DIET AND CARDIOVASCULAR DISEASE

There is growing evidence suggesting that consuming more fructose can raise the risk of cardiovascular disease (CVD) by contributing to the development of conditions such as high blood pressure, abnormal lipid levels, inflammation, and coronary heart disease (Mellor *et al.,* 2010). A high intake of fructose-sweetened beverages is associated with a 26% increase in the risk of CVD (Malik *et al.,* 2010). While the elevated risk of CVD may be partly linked to conditions like obesity or insulin resistance caused by high fructose intake, there is also a possibility of fructose-related harm specifically to the heart. This is supported by the fact that the connection between fructose consumption and an elevated risk of CVD persists even when accounting for body mass index (Malik *et al.,* 2010).

Overconsumption of a high fructose diet triggers cardiac fibrosis and inflammation by activating NF-κB signaling and toll-like receptor (TLR) 4/TLR6-IL-1R–associated kinase 4/1 (IRAK4/1) signaling mediated by differentiation 36 (CD36), leading to the activation of the NLR family pyrin domain containing 3 (NLRP3) inflammasome (Kang *et al.,* 2016). In vitro and mouse studies have demonstrated that a high fructose diet induces cardiac fibrosis and inflammation, characterized by increased levels of alpha smooth muscle-actin, collagen type I/II, and inflammatory cytokines (Xie and X, 2017). Elevated NLRP3 inflammasome activity has been identified in the atrial cardiomyocytes of atrial fibrillation (AF) patients, and mice with cardiomyocyte-specific NLRP3 expression developed spontaneous premature atrial contractions and inducible AF, which were alleviated by the NLRP3-specific inhibitor MCC950 (Yao *et al.,* 2018). Another NLRP3 inflammasome inhibitor, BAY 11-7082, also mitigated high fructose diet-induced NLRP3 inflammasome activation,

suppressing caspase-1 activity and interleukin (IL)-1β and IL-18 production in the kidney and liver (Chiazza *et al.,* 2016)

Excessive fructose consumption markedly increases the secretion of inflammatory cytokines, including transforming growth factor beta 1 (TGF-β1), tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, IL-18, and IL-6, both in the heart and serum. A high-fructose diet elevates plasma insulin levels, blood glucose, retino-binding protein 4, soluble cluster of CD36, free fatty acids, cholesterol, triglycerides, and low-density lipoprotein cholesterol, along with the inflammatory cytokines TNF-α and IL-6 (Cheng *et al.,* 2021). Therefore, excessive fructose intake can activate NF-κB/NLRP3 signaling, and the release of cytokines may contribute to cardiac inflammation and arrhythmogenesis. This suggests that targeting fructose-induced NF-κB/NLRP3 inflammasome activation and cytokine secretion could potentially mitigate cardiac dysfunction in the context of a high fructose diet and cardiovascular disease (Cheng *et al.,* 2021).

2.3 HIGH FRUCTOSE DIET AND OBESITY

Obesity is a medical condition marked by an abundance of adipose tissue and the occurrence of metabolic irregularities, including dyslipidemia, hyperglycemia, and insulin resistance (Kim et al., 2019). A primary contributor to obesity is the overconsumption of fats and sugars (Pérez-Campos *et al.,* 2020). Notably, excessive intake of fructose and saturated fats is recognized to alter the production of metabolites by the colon's intestinal microbiota, leading to systemic inflammation and disturbances in intestinal integrity (Agustí *et al.,* 2018).

The significant consumption of sugary beverages containing high levels of fructose is directly linked to the rise of obesity and its related consequences, including metabolic syndrome (Pereira *et al.,* 2017). Concurrent with the increasing incidence and

prevalence of obesity and metabolic syndrome, fructose consumption has surged by approximately 30% in the last four decades. Specifically, due to its lower ability to induce feelings of fullness and heightened palatability, fructose can lead to increased food consumption, disrupting the metabolism of lipids and carbohydrates and promoting the synthesis and accumulation of fat (Stanhope, 2016).

In modern societies, the high consumption of fructose from sugar-sweetened beverages has played a role in the onset of obesity. Sucrose and high fructose corn syrup are the primary dietary sources of fructose, undergoing metabolism in the intestine and subsequent transport into the systemic circulation. Approximately 70% of fructose intake is metabolized by the liver, with the remaining portion processed by other tissues. Key fructose transporter GLUT5 is expressed in various tissues, including adipose tissue. The accumulation of adipose tissue is now recognized as a global public health challenge. The enlargement of this tissue produces detrimental effects on the body through the secretion of various adipokines, making obesity a major risk factor for the development of metabolic syndrome and, consequently, a serious concern for overall quality of life (Cao, 2014).

In vitro experiments have revealed that fructose alone triggers adipogenesis through multiple mechanisms, encompassing;

(1) the production of triglycerides and very-low-density lipoprotein (VLDL) through fructose metabolism,

(2) the stimulation of glucocorticoid activation by enhancing 11β-HSD1 activity, and

(3) the stimulation of reactive oxygen species (ROS) production involving uric acid, NOX, and XOR expression, mTORC1 signaling, and Ang II induction.

Additionally, fructose has been noted to induce adipogenesis by upregulating ACE2 expression, leading to elevated Ang-(1-7) levels, and by inhibiting the thermogenic

program through the regulation of Sirt1 and UCP1 (Hernández-Díazcouder *et al.,* 2019).

According to epidemiological projections, it is anticipated that by 2025, approximately 18% of men and 21% of women worldwide will be classified as obese ((NCD-RisC, 2014).

The harmful effects of fructose can manifest in the early stages of life. Infants who were breastfed by mothers with a history of fructose intake during pregnancy or lactation exhibited lasting metabolic disruptions. Research by Zheng and colleagues (Zheng et al., 2016) revealed that children born to mothers who consumed fructose had higher body weight, increased food intake, elevated levels of leptin in the bloodstream, and reduced insulin sensitivity. Subsequently, Hu and collaborators (Hu *et al.,* 2010) found that each daily consumption of a glass or can of fructose-enriched beverage during childhood substantially raised the likelihood of that child developing obesity in adulthood, underscoring the significant role of fructose in the onset of obesity.

2.4 HIGH FRUCTOSE DIET AND NON-ALCOHOLIC FATTY LIVER DISEASE

Nonalcoholic fatty liver disease (NAFLD) is a persistent liver condition occurring in individuals with minimal or no alcohol consumption and without viral hepatitis. It is characterized by the buildup of surplus lipids within liver cells. NAFLD spans from simple steatosis, a generally benign condition, to nonalcoholic steatohepatitis (NASH), which has the potential to advance to cirrhosis and hepatic failure. The onset of NAFLD is typically linked with medical conditions associated with insulin resistance, such as obesity, type 2 diabetes, and dyslipidemia. It is recognized as a hepatic manifestation of metabolic syndrome (Cho *et al.,* 2021).

Increased de novo lipogenesis (DNL), the process by which hepatocellular carbohydrates are converted into fat, plays a significant role in elevating hepatic triglyceride content in nonalcoholic fatty liver disease (NAFLD) (Ameer *et al.,* 2014). Advanced techniques, including isotope methodologies and gas chromatography/mass spectrometry, have revealed the relative contributions of three fatty acid sources—adipose tissue, DNL, and dietary carbohydrates—to the accumulated fat in NAFLD. Approximately 26% of liver fat is attributed to DNL, while 15% is derived from the diet in NAFLD patients (Chung *et al.,* 2014).

A high-fructose diet can contribute to the development of NAFLD by serving as an upregulated substrate for DNL and circumventing the primary rate-limiting step of glycolysis at phosphofructokinase. The continual intake of fructose imposes a metabolic burden on the liver by inducing the expression of fructokinase and fatty acid synthase (Basaranoglu *et al.,* 2015).

2.5 HIGH FRUCTOSE DIET AND DYSLIPIDEMIA

Fructose consumption leads to acute and chronic changes in blood lipids. These changes include established features of dyslipidemia and the atherogenic lipoprotein phenotype – increased circulating triglyceride(TG) and TG-rich lipoproteins (TRL), small dense LDL (sdLDL) and changes in HDL particle composition (Hieronimus and Stanhope, 2020).

The immediate impact of fructose consumption on lipid profiles is the rise in post-meal triglycerides, noticeable within the first day of a high-fructose diet. One way this happens is through hepatic de novo lipogenesis (DNL), a process where the liver creates new lipids, especially triglycerides, from non-lipid sources like carbohydrates, including fructose. Increased fructose intake has been linked to the activation of DNL, resulting in the buildup of triglycerides in the liver and contributing to elevated levels

of circulating triglycerides (Softic *et al.*, 2016). This process intensifies with prolonged fructose consumption.

DNL is a tightly regulated process with key enzymes that are upregulated in nonalcoholic fatty liver disease (NAFLD) (Paglialunga *et al.,* 2016). Dietary fructose amplifies the levels of these enzymes in NAFLD because fructose, absorbed through the portal vein, reaches the liver in higher concentrations than other tissues. Unlike glucose metabolism, fructose breakdown produces metabolites that activate hepatic DNL. High-fructose diets increase ChREBP expression, a transcription factor for lipogenesis, and enhance fructose-induced glucose production independently of insulin signaling (Kim *et al.,* 2016).

In obesity, dyslipidemia manifests with elevated fasting and postprandial triglycerides (TG) alongside a predominance of small dense low-density lipoprotein (LDL) and low high-density lipoprotein cholesterol (HDL-C) levels. Hypertriglyceridemia plays a significant role in causing other lipid abnormalities due to delayed clearance of triglyceride-rich lipoproteins, leading to the formation of small dense LDL-C (Klop *et al.*, 2013).

In obesity, lipolysis of triglyceride-rich lipoproteins is hindered by reduced mRNA expression levels of lipoprotein lipase (LPL) in adipose tissue, diminished LPL activity in skeletal muscle, and competition for lipolysis between very low-density lipoprotein (VLDL) and chylomicrons. Increased postprandial lipemia results in elevated levels of free fatty acids (FFA), causing detachment of LPL from its endothelial surface. LPL may remain attached to VLDL and intermediate-density lipoprotein (IDL), further depleting triglycerides. The exchange of triglycerides from these remnants for cholesterol-esters from HDL by cholesteryl ester transfer protein (CETP), along with hepatic lipase action, leads to the formation of small dense LDL.

In the presence of hypertriglyceridemia, the cholesterol-ester content of LDL decreases, while the triglyceride content increases due to CETP activity. However, increased triglyceride content within LDL is hydrolyzed by hepatic lipase, resulting in the formation of small, dense LDL particles. The development of small dense LDL in obesity is primarily due to increased triglyceride concentrations and is independent of total body fat mass. Small dense LDL particles have a longer residence time, enhancing their atherogenicity (Klop *et al.*, 2013).

Chylomicron remnants and LDL may migrate into the sub-endothelium and become trapped, where they can be taken up by monocytes/macrophages. Small dense LDL have a heightened affinity for arterial proteoglycans, leading to increased subendothelial lipoprotein retention. However, subendothelial remnants of chylomicrons and VLDL do not require modification for uptake by scavenger receptors of macrophages, unlike native LDL. Small dense LDL are more prone to oxidation, partly due to lower free cholesterol and anti-oxidative content. The size of lipoprotein particles is crucial for their migration through the endothelium, with LDL particles migrating more easily than chylomicron remnants. However, the number of migrated particles does not necessarily correlate with cholesterol deposition, as chylomicron remnants contain approximately 40 times more cholesterol per particle than LDL. Alternatively, LPL-enriched remnants of chylomicrons and VLDL may be transported to tissues where interaction with proteoglycans and lipoprotein receptors leads to particle removal. This process occurs mainly in the liver, acting as an anti-atherogenic mechanism. However, it may also occur in other tissues where cholesterol cannot be efficiently removed, leading to cholesterol accumulation and initiation of atherosclerotic plaque formation (Klop *et al.*, 2013).

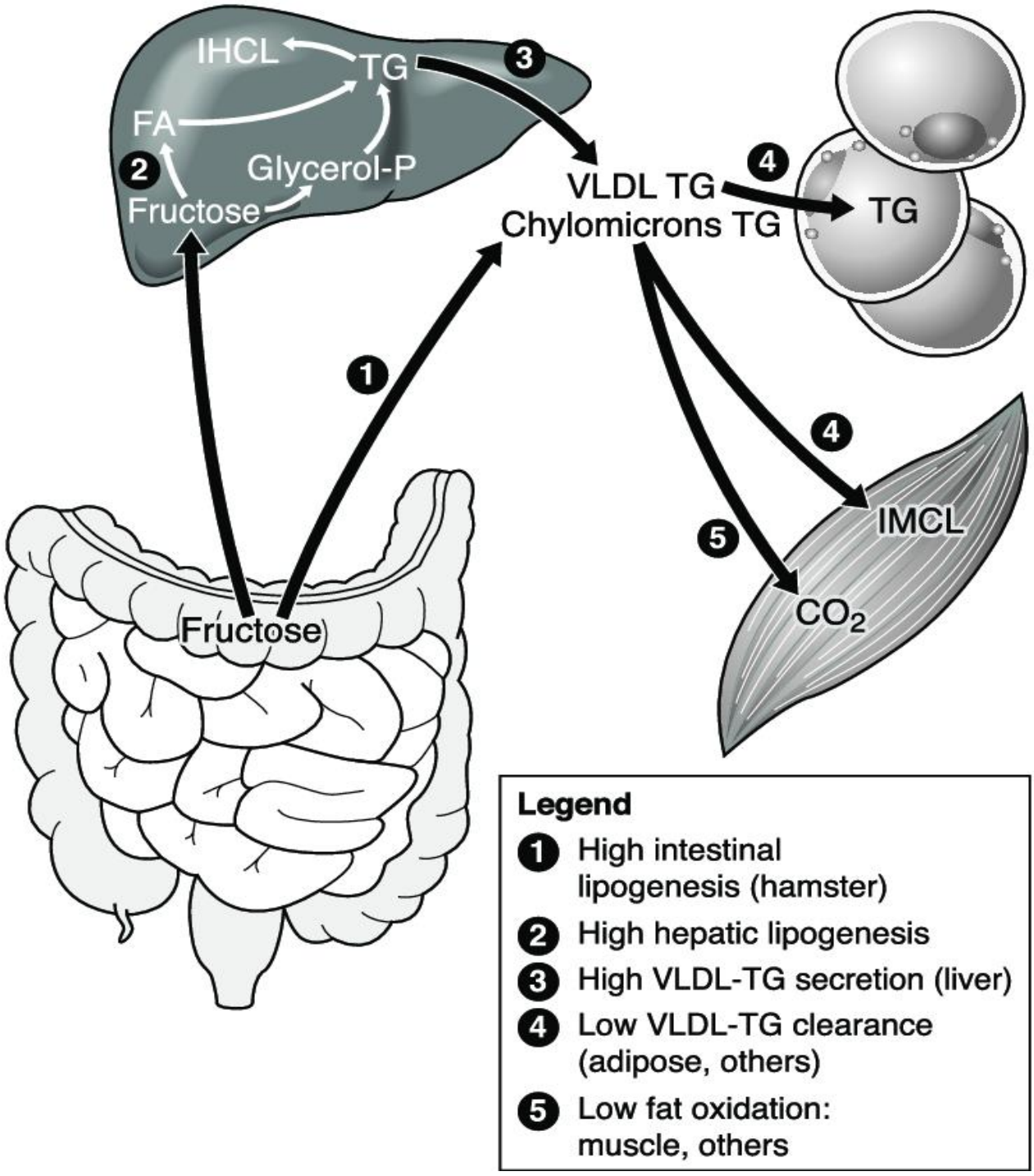

Figure 2.2: Fructose induced dyslipidemia (Tappy *et al*., 2010).

2.6 LIPIDS

Lipids are crucial compounds with fatty, waxy, or oily characteristics, playing essential roles in various bodily functions and acting as fundamental components for all living cells. They contribute to hormone regulation, nerve impulse transmission, organ cushioning, and serve as a storage form for energy in the body, stored as fat.

The primary lipid categories include phospholipids, sterols (comprising different cholesterol types), and triglycerides, with the latter representing over 95% of lipids found in food. Elevated concentrations of lipids are present in fried foods, animal fats, and certain dairy products such as cream, butter, and cheese (Muro *et al.,* 2014).

Cholesterol and triglycerides, major lipids in humans, are transported in the blood by lipoproteins. When the liver secretes a lipoprotein into the plasma, it consists of cholesterol, triglycerides, and a single apolipoprotein B100 molecule (apoB), and is known as a very low-density lipoprotein (VLDL). Lipoprotein lipase rapidly removes triglycerides from VLDL for energy consumption and storage. As triglycerides are progressively removed, the lipoprotein transforms into a VLDL remnant particle. After most triglycerides are removed, the lipoprotein becomes denser and is termed a low-density lipoprotein (LDL) (Ference *et al.,* 2020).

Plasma lipoproteins are categorized into seven classes determined by their size, lipid composition, and apolipoproteins. These classes include chylomicrons, chylomicron remnants, very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and lipoprotein-a (Lp-(a)) (Feingold *et al.,* 2021).

Chylomicrons; Chylomicrons play a crucial role in transporting fatty acids from the intestine, along with a small amount of cholesterol. Dietary triglycerides undergo

breakdown by pancreatic lipase in the duodenum. The intestine absorbs free fatty acids, monoglycerides (composed of one glycerol and one fatty acid), and some diglycerides. Subsequently, the enterocyte reconstructs these components into triglycerides, combining them with dietary cholesterol, apoB48, apo A I and II, apo CI, CII, and CIII to form chylomicrons. The enterocyte releases chylomicrons into the lymph system, which then flows into the inferior vena cava, ultimately entering the bloodstream (Robinson, 2024).

Chylomicron remnants; Lipoprotein lipase acts on chylomicrons in peripheral tissues, leading to the reduction of triglycerides and the formation of smaller particles known as chylomicron remnants. These remnants have a higher concentration of cholesterol compared to chylomicrons and possess pro-atherogenic properties (Julve *et al.,* 2016).

Very low density lipoprotein (VLDL); VLDLs, while also rich in triglycerides, have lower triglyceride content and higher cholesterol content compared to chylomicrons. The protein composition of VLDL differs from chylomicrons, with full-length apo B (apo B100) being the major structural protein instead of the truncated apo B48 form. Similar to chylomicrons, VLDLs undergo triglyceride removal by lipoprotein lipase. Their role is to transport triglycerides synthesized in the liver and intestines to capillary beds in adipose tissue and muscle. Here, triglycerides are hydrolyzed to provide fatty acids for energy production or can be stored as fat if not immediately needed. After triglyceride removal, VLDL remnants (IDLs) can be further metabolized into LDL. VLDLs also participate in cholesterol transfer from HDL, contributing to the inverse relation between HDL cholesterol and VLDL triglyceride. This transfer is facilitated by cholesterol ester transfer protein (CETP). Elevated VLDL levels are commonly observed in type 2 diabetes, and the

causes of this abnormality will be discussed in the section on diabetic lipid abnormalities (Mason *et al.,* 2010).

Intermediate density lipoprotein (IDL); The extraction of triglycerides from VLDL by muscle and adipose tissue leads to the creation of IDL particles, which have a higher concentration of cholesterol. These particles include apolipoprotein B-100 and E. IDL particles are associated with promoting atherosclerosis (Krauss and King, 2023).

Low density lipoprotein (LDL); LDL represents the final stage in the progression from VLDL and serves as the primary carrier of cholesterol throughout the body. LDL receptors located on the liver adhere to the apoB-100 present in LDL particles. This complex undergoes endocytosis into the cell, where the LDL is broken down while preserving the LDL receptor. Subsequently, the LDL receptor returns to the cell surface through a recycling process that can be repeated up to 150 times. The activity of Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) plays a crucial role in regulating the LDL receptor. PCSK9 forms an irreversible bond with the LDL receptor-LDL complex, leading to the degradation of both the LDL receptor and LDL (Robinson, 2024).

High density lipoprotein (HDL); HDL play a crucial role in the reverse transport of cholesterol from peripheral tissues to the liver, potentially making HDL anti-atherogenic. Moreover, HDL particles possess properties that are anti-oxidant, anti-inflammatory, anti-thrombotic, and anti-apoptotic, which may contribute to their ability to prevent atherosclerosis. HDL particles are rich in cholesterol and phospholipids, and they are associated with apolipoproteins A-I, A-II, A-IV, C-I, C-II, C-III, and E. Apo A-I serves as the core structural protein, and a single HDL particle may contain multiple Apo A-I molecules. Additionally, mass spectrometry has

identified proteins linked to proteinase inhibition, complement activation, and the acute-phase response associated with HDL particles (Asztalos *et al.,* 2019).

Lipoprotein-a (Lp-(a)); Lp(a) is a type of LDL particle that is distinguished by the presence of apolipoprotein(a) linked to Apo B-100 through a disulfide bond. Lp(a) consists of Apo(a) and Apo B-100 in a 1:1 molar ratio, and the size of Lp(a) particles can vary significantly depending on the size of apolipoprotein(a). This particle is associated with promoting atherosclerosis (Schmidt *et al.,* 2016).

2.7 LIPID PROFILE

Lipid profile, also known as lipid panel is a blood test designed to assess the levels of specific fat molecules, known as lipids, in your blood. Typically, the panel comprises four cholesterol measurements and a triglyceride measurement. Elevated levels of lipids (cholesterol and triglycerides) in the blood can result in the accumulation of these substances in blood vessels and arteries, potentially leading to damage and an increased risk of cardiovascular issues.The National Heart, Lung, and Blood Institute (NHLBI) suggests that individuals should undergo their initial cholesterol screening between the ages of 9 and 11, with subsequent screenings every five years. For men aged 45 to 65 and women aged 55 to 65, cholesterol screenings are recommended every 1 to 2 years. Individuals over the age of 65 are advised to have annual cholesterol tests (Mayo Clinic, 2024). As a result, healthcare professionals utilize lipid panels for individuals of all ages, including children and adults, to assess the likelihood of cardiovascular diseases such as heart disease, heart attack (myocardial infarction), and stroke (Cleveland Clinic, 2021).

A lipid panel, a specific blood test, measures five different types of lipids in a blood sample. These lipids encompass:

1. **Total cholesterol:** This represents the overall cholesterol content in the blood (Mayo Clinic, 2024).

2. **Low-density lipoprotein (LDL) cholesterol:** Often referred to as the "bad" cholesterol, an excess of LDL can lead to the formation of fatty deposits in arteries, reducing blood flow and increasing the risk of heart attack or stroke (Mayo Clinic, 2024).

3. **Very low-density lipoprotein (VLDL) cholesterol:** Low levels of VLDL cholesterol are typically detected in blood samples obtained after fasting. In a fasting sample, elevated VLDL cholesterol levels could be a sign of aberrant lipid metabolism (Cleveland Clinic, 2021).

4. **High-density lipoprotein (HDL) cholesterol:** Known as the "good" cholesterol, HDL helps transport away LDL cholesterol, maintaining open arteries and promoting smoother blood flow (Mayo Clinic, 2024).

5. **Triglycerides:** These are a type of fat in the blood formed when the body converts excess calories into triglycerides, which are stored in fat cells. Elevated triglyceride levels can be associated with factors such as being overweight, consuming too many sweets or excessive alcohol, smoking, leading a sedentary lifestyle, or having diabetes with high blood sugar levels (Mayo Clinic, 2024).

2.8 YOYO CLEANSER BITTERS

2.8.1 OVERVIEW OF YOYO CLEANSER BITTERS

The World Health Organization (WHO) defines herbal medicine as the utilization of any plant part for therapeutic purposes or as precursors for drug synthesis. Herbal remedies, with a history spanning diverse cultures, were the primary form of treatment before modern medicine. Over the past decades, there has been a significant global interest in self-medicating with natural therapies, particularly herbal medicine. Herbal supplements are commonly consumed without medical consultation, driven by the perception that natural products are inherently safe due to

their historical use and the accessibility and affordability of these products. However, the WHO reports that the global use of herbal medicine exceeds that of conventional therapies by two to three times . Despite the long-standing use of herbal supplements, their historical use does not guarantee safety, especially with prolonged use, high doses, or concurrent use with other medications or health conditions (Timipa *et al.,* 2021). Traditional herbal medicine, widely used for thousands of years, initially took the form of crude drugs such as tinctures, teas, poultices, and powders. Herbal medicine continues to be a primary form of healthcare for approximately 75–80% of the global population, particularly in developing countries (Odunola *et al.,* 2021).

All organic drugs in Nigeria are currently acknowledged by the national regulatory body, the National Agency for Food and Drugs Administration and Control (NAFDAC). Yoyo Cleanser Bitters, categorized as an organic drug in the herbal bitters class, was introduced to the market in 2003 by Abllat Nigeria Company Limited, a local manufacturer of healthcare products. Since its launch, it has gained widespread acceptance and usage among the general population, becoming a household name. Its popularity can be attributed to its perceived safety and effectiveness, with minimal reported adverse reactions following administration. Yoyo Cleanser Bitters belongs to the category of internationally acclaimed bitters and is formulated with five herbal constituents, including Aloe vera (True aloe, Lily of the forest), Acinos Arvensis (Basil thyme), Citrus aurantifolia (Bitter orange), Chenopodium murale (Nettleleaf goosefoot), and Cinamomum aromaticum (Cassia) (Anionye *et al.,* 2017).

Figure 2.3: Yoyo Cleanser Bitters.

Source: Flavor spice LLC, 2024.

2.8.2 COMPONENT OF YOYO CLEANSER BITTERS

Citrus aurantifolia: C. aurantifolia is a small herbaceous plant known for its unique scent. Its fruit is somewhat round with a tapered end, offering a very sour, juicy flavor and a potent aroma. This plant is commonly utilized in various applications, including as a raw material for cosmetics, a food flavoring, a beverage enhancer, and a component in traditional medicine (Indriyani *et al*., 2023) Research has demonstrated that C. aurantifolia exhibits a range of biological activities, including insecticidal, larvicidal, and repellent properties (Galovičová *et al.,* 2022); antioxidant, anticancer, and antimicrobial effects (Julaeha *et al.,* 2022) ; as well as antiseptic, antiviral, antifungal, astringent, anticholesterol, diuretic, appetite-stimulating, and constipation-relieving properties. Additionally, it has anti-inflammatory and analgesic activities. These diverse biological functions are attributed to the secondary metabolites present in C. aurantifolia, such as alkaloids, coumarins, flavonoids, carotenoids, phenolics, terpenes, limonoids, and essential oils. The concentration and efficacy of these metabolites can be affected by various factors, including physicochemical properties, soil composition, sun exposure, geographical location, and the specific part of the plant utilized.

Acinos Arvensis: commonly known as basil thyme or mother-of-thyme, is a small perennial herb belonging to the Lamiaceae family. This plant is native to Europe and parts of Asia, typically found in dry, rocky, and sunny locations such as grasslands and hillsides. It features small, aromatic leaves and produces clusters of purple or pink flowers during the summer (Smith and P.M., 2022).

Acinos arvensis is valued for its pleasant thyme-like fragrance and is often used in traditional medicine for its potential therapeutic properties. The herb is believed to have antiseptic, astringent, and carminative effects. Additionally, it can be used as a

culinary herb to flavor various dishes. Its essential oils and extracts are also explored for their antimicrobial and antioxidant activities, contributing to its application in natural remedies and wellness products (Smith and P.M., 2022).

Chenopodium murale: commonly known as nettleleaf goosefoot, serves various functions owing to its medicinal, culinary, and ecological properties.

Medicinally, Chenopodium murale has been traditionally used as a natural remedy for several health issues. The plant's diuretic properties make it valuable in promoting urine production and aiding in the elimination of toxins from the body. Additionally, it has been employed to alleviate digestive ailments such as indigestion, bloating, and constipation. Culinarily, both the leaves and seeds of Chenopodium murale are edible and offer a rich source of nutrients. The young leaves can be consumed raw or cooked and are often used as a leafy vegetable in salads or cooked dishes. The seeds, known as pseudo-grains, are rich in protein, fiber, and essential minerals, making them a nutritious addition to various recipes, including bread, porridge, and soups (Kumar *et al.*, 2019).

Cinamomum aromaticum: derived from the dried inner bark of an evergreen tree, is the predominant type of cinnamon found in North American markets. It contains compounds believed to enhance insulin sensitivity, potentially aiding in blood sugar regulation for individuals with diabetes. Additionally, one of its key components, cinnamaldehyde, is thought to possess antibacterial and antifungal properties.

Widely utilized as a spice and flavoring agent in culinary applications, cassia cinnamon is also employed medicinally, particularly in the management of diabetes. Despite its popularity for addressing conditions like prediabetes and obesity, scientific evidence supporting these uses remains limited (Gutierrez *et al.*, 2016).

2.8.3 PHYTOCHEMICAL COMPONENT OF YOYO CLEANSER BITTERS

Yoyo bitters contain saponins, flavonoids, anthocyanins, cardiac glycosides, and terpenoids. A quantitative study conducted by Paula-Peace et al. (2021) found that Yoyo bitters also include phenols, flavonoids, flavanols, tannins, and carotenoids. The research into the phytochemical composition of yoyo cleanser bitters revealed that phenols are the most abundant component, while carotenoids are the least prevalent, alongside the presence of steroids and carbohydrates (Paula-Peace et al., 2021).

Phenolic compounds are an extensive group of plant secondary metabolites found in nearly all plants in varying amounts. This chemically diverse group includes significant derivatives such as flavonoids, tannins, lignins, and anthocyanins (Kumar *et al.*, 2022). Their structures range widely, from simple phenolic acids to complex polyphenols like flavonoids, which contain multiple functional groups.

Phenolic and flavonoid compounds are recognized for their potent antioxidant abilities, achieved through diverse mechanisms. Phenolic compounds' hydroxyl groups serve as effective hydrogen donors, swiftly reacting with reactive oxygen and nitrogen species to halt the generation of new free radicals. Additionally, these compounds can chelate metal ions involved in free radical formation, further bolstering their antioxidant effects. Apart from their antioxidant prowess, phenolic compounds found in dietary supplements exhibit anticancer properties and provide protection against cardiovascular diseases and various autoimmune conditions (Kumar *et al.*, 2022).

Flavonoids, a major subset of phenols, display a broad spectrum of chemical and pharmacological characteristics. They possess antibacterial, cytotoxic, anti-inflammatory, hormone-modulating, anti-allergic, immune-boosting, diuretic, anti-spasmodic, hypocholesterolemic, hepatoprotective, and vascular benefits. Notably,

flavonoids also demonstrate robust antioxidant activities, effectively scavenging various free radicals and mitigating lipid peroxidation. These multifaceted properties render them valuable in medicinal applications (Tungmunnithum et al., 2018).

Tannins are polyphenolic compounds found in plants and are known for their diverse pharmacological benefits, including anti-inflammatory, antioxidant, anti-tumor, anti-infective, blood clotting, hypolipidemic, immunomodulatory, and antimicrobial properties (James-Okoro *et al.,* 2021).

Saponins have been reported to possess wound-healing, hypocholesterolemic, hemolytic, antimicrobial, anti-inflammatory, immunostimulant, and blood clotting properties. Terpenoids exhibit antimicrobial, diuretic, anti-cancer, anti-ulcer, anti-malarial, hepaticidal, and anti-inflammatory activities (James-Okoro *et al.,* 2021).

Cardiac glycosides are known for their cardiotonic, anticancer, and antineoplastic effects and are commonly used in the treatment of heart diseases (James-Okoro *et al.,* 2021).

2.9 ATORVASTATIN

Atorvastatin, a member of the statin class, represents a pharmacological triumph in the realm of cardiovascular medicine. Renowned by its brand name Lipitor, this drug has redefined the landscape of cholesterol management and cardiovascular risk reduction.The U.S. Food and Drug Administration (FDA) approved this medicine in 1996. HMG-CoA reductase inhibitors, commonly known as statins, play a crucial role in lowering lipid levels and are employed in the primary, secondary, and tertiary prevention of coronary heart disease. Atorvastatin, a member of this class, competitively inhibits the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. By impeding the conversion of HMG-CoA to mevalonate, atorvastatin effectively reduces cholesterol synthesis in the liver. Additionally, it

enhances the presence of low-density lipoprotein (LDL) receptors on hepatic cells, further contributing to cholesterol regulation (McIver and Siddique, 2024).

Administration; Atorvastatin is accessible in the form of tablets containing atorvastatin calcium, with strengths ranging from 10 to 80 mg. The administration of this medication is flexible, allowing for consumption with or without food, preferably at the same time daily. While statins are commonly advised to be taken at bedtime due to the cyclical nature of endogenous cholesterol synthesis, where production peaks during fasting, the longer half-life of atorvastatin provides increased flexibility in choosing dosing times compared to shorter half-life statins such as lovastatin, fluvastatin, and simvastatin (McIver and Siddique, 2024).

Figure 2.4: Atorvastatin Tablets

Source: Asset pharmacy (2024).

Mechanism of action; Atorvastatin competitively inhibits the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, an integral step in cholesterol synthesis. By obstructing the conversion of HMG-CoA to mevalonate, atorvastatin effectively reduces cholesterol production in the liver. Moreover, it enhances the presence of low-density lipoprotein (LDL) receptors on hepatic cells.

Clinical evidence supports the efficacy of atorvastatin in managing various lipid disorders, including familial hypercholesterolemia, mixed dyslipidemia, isolated hypertriglyceridemia, and nonfamilial hypercholesterolemia. Across these conditions, atorvastatin has demonstrated its capacity to decrease total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), apolipoprotein B (apo B), very-low-density lipoprotein (VLDL-C), and triglycerides (TGs). Simultaneously, it exhibits a positive impact by increasing high-density lipoprotein cholesterol (HDL-C). Notably, in cases of dysbetalipoproteinemia, atorvastatin has proven effective in reducing intermediate-density lipoprotein (IDL-C) (McIver and Siddique, 2024).

- Absorption

Upon oral intake, atorvastatin undergoes rapid absorption, achieving its highest plasma concentration within the first 1 to 2 hours. However, its bioavailability is restricted to a mere 14%, primarily attributed to extensive first-pass metabolism.

- Distribution

Atorvastatin exhibits high binding to plasma proteins, surpassing 98%, and displays a volumetric distribution of approximately 380 liters.

- Metabolism

The process of metabolizing atorvastatin involves its conversion by cytochrome P450 3A4 (CYP3A4) into active ortho- and para-hydroxylated metabolites.

- Excretion

Atorvastatin, along with its metabolites, is excreted through bile, and there is no evidence of enterohepatic recirculation for atorvastatin. The half-life of atorvastatin is approximately 14 hours, while its active metabolites exhibit a longer half-life ranging from 20 to 30 hours.

Contraindications; Contraindications for atorvastatin encompass individuals with hypersensitivity to any of its constituents. Additionally, active liver disease is considered a contraindication, although the potential benefits of using lipid-lowering therapy in chronic liver conditions, such as non-alcoholic fatty liver disease and hepatitis, may outweigh potential risks.

Furthermore, atorvastatin is contraindicated in pregnant women or those planning pregnancy, and all women of childbearing age should receive counseling regarding potential fetal risks if they conceive while on atorvastatin. To mitigate risks, current guidelines suggest discontinuing statin therapy for at least 3 months before attempting pregnancy, particularly during the first trimester. If a patient becomes pregnant, immediate discontinuation of atorvastatin is advised. Female patients are also advised to refrain from atorvastatin while breastfeeding. If atorvastatin therapy is deemed necessary, patients should be directed to discontinue breastfeeding (McIver and Siddique, 2024).

Adverse effects; Patients taking atorvastatin commonly experience adverse effects such as arthralgia, dyspepsia, diarrhea, nausea, nasopharyngitis, insomnia, urinary tract infection, and pain in the extremities.

Notably, myopathies, characterized by symptoms like muscle aches, tenderness, or weakness, and elevated creatine phosphokinase levels exceeding ten times the upper limit of normal, have been reported in individuals using atorvastatin. Instances of

rhabdomyolysis, a severe condition involving muscle breakdown, have also been documented with atorvastatin use. Patients with impaired renal function may face an elevated risk of developing rhabdomyolysis. The concurrent use of atorvastatin with other medications that increase its plasma concentrations heightens the risk of myopathies and rhabdomyolysis. Atorvastatin can cause liver function test abnormalities. If patients develop serum transaminases over 3 times the upper limit of normal, plasma concentrations require more frequent monitoring until normalized or atorvastatin therapy should undergo dose reduction or be discontinued (Filppula *et al.,* 2021). Management strategies for statin-induced myopathies include temporarily discontinuing therapy, switching to an alternative statin, or reducing the dose (McIver and Siddique, 2024).

CHAPTER THREE

MATERIALS AND METHODS

3.1. MATERIALS:

3.1.1. Test materials

Yoyo bitters was purchased from pharmaceutical stores opposite the University of Benin Teaching Hospital (UBTH), Ugbowo Lagos Road, Benin City, Edo State, Nigeria.

3.1.2. Equipment

Apparatus and equipments	Producer/maker
Beakers (50, 150 and 250ml)	Pyrex (England)
Retort Stand	
Tripod Stand, Bunsen Burner and Gas Supply	
Pippettes (1,10 and 25ml)	Pyrex (England)
Automated micropippette (0-100 µl, 0-1000µl).	Micropet and Accumax PRO.
Conical flasks.	Pyrex (England)
Filter paper (0.45µm and 125mm)	Whatman (England)
Cuvettes	Pyrex (England)
Needles and syringes (1ml, 2ml, 5ml, 10ml)	
Paper tapes, cardboard papers and pins	

Cotton wool and Methylated spirit	
Animal cages	UNIBEN MEDBCH Dept. (Nigeria)
Oro-gastric Gavage	UNIBEN MEDBCH Dept. (Nigeria)
Stop watch	
Test tube racks and test tubes	UNIBEN MEDBCH Dept. (Nigeria)
Volumetric flasks (100, 250 and 500ml)	Technics (England)
HH-W Constant Temperature Water Bath	B. Bran Sc. Inst. Company, England.
Analytical weighing balance	Metter H-80 (Germany)
Water distiller	B. Bran Sc. Inst. Company, England.
Simple Weighing Balance	Adventurer OHAUS AR1530
T70UV/VIS Spectrophotometer	PG Instruments Ltd., UK.
microplate reader	PG Instruments Ltd., UK.
Refrigerator	Citizens PRC4246
80-2 model Electric Centrifuge.	B.Bran Scientific and Instrument Company, England

3.1.3 Chemicals/Reagents:

Reagent/Enzyme kits and other reagents used were of standard quality and were purchased from qualified/accredited dealers/suppliers or their manufacturers' representative in Nigeria. The Chemicals used were of analytical grade and an accredited dealer - Pyrex Laboratories, Benin, Nigeria. The process for the preparation or reconstitution of some of the reagents of this study are as shown in Appendix I.

3.1.4 Reagents/chemicals used for the assessment of lipid profile:

a. Total cholesterol:

The total cholesterol level is determined using the (Randox Lab.UK; purchased from manufacturer's representative in Nigeria). The kit contains the following reagents:

Cholesterol standard {(5.04mmol/l or 195mg/dl)} and **Solution R1 -** 4-aminoantipyrine (0.30mm/l), Phenol (6mmol/l), Peroxidase (≥0.5IU), Cholesterol esterase (≥0.15IU), Cholesterol oxidase (≥0.1IU), Pipes buffer (80mmol/l pH 6.8).

b. HDL-cholesterol:

The HDL-cholesterol level is determined using the (Randox Lab.UK; purchased from manufacturer representative in Nigeria).

The kit contains the following Reagents:

HDL-cholesterol standard (5.04mmol/l or 195mg/dl); **Precipitant R1**. Phosphotungstic acid (0.55mmol/l), Magnesium chloride (25mmol/l); **CHOL Reagent**.

c. Triglyceride:

The triglyceride level is determined using the (Randox Lab.UK; Purchased from manufacturers representative in Nigeria). The kit contains the following reagents:

Standard (2.21 mmol/l or 196mg/dl); **R1a- Buffer** (Pipes Buffer 40mm/l, pH 7.6; 4-chlorophenol 5.5mmol/l; magnesium ions 17.5mm/l); **R1b-Enzyme reagent** (4-aminophenazone 0.5mm/l; ATP 1.0; Lipases ≥ 150IU; Glycerol kinase≥0.4IU; Glycerol-3-phosphate oxidase ≥1.5IU; Peroxidase ≥0.5IU).

3.2.0 METHODS

3.2.1. Animals for the study.

The experimental animals for this study were handled in strict compliance with international guidelines as prescribed by the Canadian Council on the care and use of laboratory animals in biomedical research (1984). The research was approved as complying with research ethics by the Research Ethics Committee of the College of Medical Sciences, University of Benin, prior to the commencement of the research (see approval certificate in appendix II). 20 male *Wistar* rats were obtained from the Anatomy Department, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria. The rats were housed in a well-ventilated room, in wooden cages with wire-mesh floor and top, in the animal house of the Department of Medical Biochemistry, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria. The temperature of their environment ranged between 25-29^{0}C and they were allowed the recommended diurnal 12-hr light and dark cycle. They were fed ad-libitum with standard pelleted grower's mash (basal diet) and clean tap water for two weeks to allow them time to acclimatize.

3.2.2. Experimental Diets.

The control or basal diet was a standard pelleted grower's mash. The experimental diet (metabolic syndrome-inducing diet) was made by adding to a proportionate quantity of the basal diet, 60% of white crystalline powdered D (-) Fructose (Abdelrahman *et al.,* 2018). This metabolic syndrome-inducing diet was supported by 10% fructose drinking water (Lirio *et al.,* 2016).

The final composition of both the basal and experimental diets are indicated in Table 1 and 2

Table 1: Composition of the basal diet (g/1000g) based on of the standard pelleted growers mash of Jerrison Agro Allied Services, Benin City, Nigeria.

Ingredients	**Basal diet (g)**
Maize	280.0
Wheat Offal	280.0
Palm Kernel Cake	208.0
Soyabean Meal	48.0
Groundnut Cake	100.0
Fish Meal (65%)	12.0
Lysine	1.6
Bone Meal	12.0
Limestone	52.0
Methionine	0.8
Grower Premix	2.4
Salt	3.2
Total	1000.0

Table 2: Composition of the 60% high fructose diet salt (metabolic syndrome-inducing) diet (g/1000g) (Abdelrahman *et al.,* 2018).

Ingredients	**High-Fructose Diet (g)**
Basal diet in Table 1	400.0
Pure white crystalline powdered Fructose	600.0
Total	1000.0

3.2.3. Experimental Design and Feeding Protocol

Twenty (20) male *Wistar* rats weighing between 200 - 220g, were used for this study. **A**fter 14 days acclimatization of the 20 animals, they were weighed and randomly divided into four (4) groups of five (5) rats each, with the weights of those in a group being representative of the weight range of all the rats, such that the average weight of all the groups at the onset of the experimental period was 220.0±1.0g. The rats were fed ad-libitum during the entire course of the 8-weeks (56 days) study, according to the feeding protocol assigned for each group as specified in the feeding protocol below, based on the assigned diets which include the basal diet (standard pelleted mash) and the high-fructose diet, the different doses of the herbal bitters, the drug atorvastatin and clean tap-water. They were allowed the recommended 12-hr light and dark cycle. Care was taken to determine the quantity of feed consumed daily. They were housed in wooden cages with a tiny-wire meshed/iron gauze flooring to allow the rat-excreta to be collected into another tray receptacle below covered with a bedding material, to prevent coprophagy. The cages, their surroundings, the receptacle tray below with its bedding, were cleaned and disinfected daily.

The animals were given orally, clean tap water (for the control groups), 10% fructose drinking water (for all other groups), moderate doses of the drug, and a high dose of the Yoyo Cleanser bitters, using an oro-gastric gavage, according to the equivalent dose (to the weight of the rats) of the effective dose already prescribed for man. **Earlier studies have already established the toxicity (LD_{50}) and dose range that can be used in rats, in reference to the bitters of this study (Anionye *et al.*, 2015; 2017a).** The animals were observed for signs of toxicity and mortality.

Protocol:

Control groups

- **Group 1:** Basal diet + clean tap water for 8 weeks (normal control)
- **Group 2:** High fructose diet + 10% fructose-water for 8 weeks
- **Group 3:** High fructose diet + 10% fructose-water + Atorvastatin (0.57 mg/kg-bw) for 8 weeks

Experimental group

- **Group 4:** High fructose diet + 10% fructose-water + Yoyo bitters (1200 mg/kg) for 8 weeks.

3.2.4. Dosage Regimen for the Herbal Bitters

The manufacturer's recommendation and prior studies have established an effective dose for the herbal bitters for an adult man to be an average 40ml daily as a single dose or in two doses (Mendie, 2009; Anionye *et al.,* 2015, 2017a,b,c; Anionye and Onyeneke 2016 a,b,c,d,e). The manufacturers of the herbal bitters of this study recommend that an average of 40ml of the herbal bitters per day, can be consumed by an adult man, usually assigned a physiological weight of 70kg. Doubling the dose to 80ml is therefore high and necessary in some diseased conditions. Appropriate calculations was done to determine the initial equivalent doses of the bitters (distilled water in the case of the control groups) in ml/kg mean body weight of the rats to be given to each group. As the initial mean body weights of rats in each group at the beginning of the study was about 220g, the equivalent volume [in millilitres-(ml)] of the bitters/distilled water that was given to the rats was calculated as shown in

appendix I:

This amounted to: 1200 mg/kg (0.15 ml for a 220g rat).

3.2.5. Dosage Regimen for the Drug

Following the same principles as adopted for the dosage of the herbal bitters

a. Dosage regimen for atorvastatin: (EMDEX, 2007a)

40mg is consumed by a 70,000g man (70kg)

This amounts to 0.126mg for a 220g rat (meaning a dose of 0.126mg/220g = approx. $0.57x10^{-3}$mg/g of rat or 0.57mg/kg of rat body weight) (appendix III).

3.2.6. Weekly Body Weight:

The body weight of each rat was assessed using a sensitive balance during the acclimatization period, once before commencement of dosing (day 1), once weekly during the dosing period, (day 7, 14, 21, 28, 35, 42, 49, 56) and once on the day of sacrifice (day 56). (Aniagu *et. al.,* 2005). The results was used to determine the average weekly weight of the rats in each group, after which the weekly weight of the bitters fed rats and the control rats was used to determine the weekly dose of the bitters to be given to the rats and the **weight gain or lost weekly** by the rats.

3.2.7. Daily Quantity of Feed Consumed:

The quantity of feed given to each group of rats daily was determined by subtracting the quantity of feed left the next morning from that given the day earlier. From the results, the average quantity consumed per day for the period of the study, by the rats, was determined. This quantity of feed consumed by each rat was assessed using a sensitive balance from the commencement of dosing (day 1), until the day of sacrifice

(day 56) (Aniagu et. al., 2005). The results was used to determine the quantity of feed consumed per week.

3.2.8. Feed efficiency:

This was determined using the formula:

$$\text{Feed efficiency} = \frac{\text{Weight gain or lost/day/rat}}{\text{Feed consumed/day/rat}} \times 100$$ (Matos et al., 2005)

3.2.9. Observations (symptoms, signs and mortality):

The animals were observed for toxic symptoms such as weakness or aggressiveness, food refusal, loss of weight, diarrhoea, discharge from the eyes and ears, noisy breathing and other physiological changes including mortality, (Fielding and Metheron, 1991; Vijoyalakshmi et al, 2000).

Clinical signs that were assessed before dosing, immediately and 4hrs after dosing, include level of sedation, restlessness, changes in nature of stool, urine and eye colour, excretion of worms, diarrhoea, haematuria, uncoordinated muscle movements, etc. (Fielding and Metheron, 1991; Vijoyalakshmi et al, 2000).

3.2.10. Blood Sample Collection and Preparation:

Three specimen bottles was used for collection of blood from each animal. Anticoagulant bottles containing **lithium heparin** for assay of parameters for lipid profile status.

The last dose of the respective diets, drugs and bitters was administered on morning of the 56th day. All meals where stopped by 7pm on the 56th day. After an overnight fast, blood samples were collected from the animals (following chloroform anaesthesia and sacrifice/opening up of the animals), using syringes and needles via the inferior vena cava and cardiac puncture, and put into already labelled lithium

heparin without undue pressure to either the arm or the plunger of the syringe. The samples were then mixed by gentle inversion.

The samples in the lithium heparin where centrifuged at 4000r/min for 10mins to obtain plasma. The serum supernatants were separated into **sterile plain bottles** and used for assay of the required biochemical parameters.

3.2.11. Assessment of the lipid profile

Determination of Plasma Total Cholesterol (Trinder, 1969).

Assay Principle

The cholesterol was determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase (Trinder, 1969).

$$\text{Cholesterol ester} + H_2O \xrightarrow[\text{esterase}]{\text{cholesterol}} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow[\text{oxidase}]{\text{cholesterol}} \text{Cholestene} - 3\text{-one} + H_2O_2$$

$$2H_2O_2 + \text{phenol} + \text{4-aminoantipyrine} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4H_2O$$

Procedure:

The following were pipetted into curvette:

	Reagent blank (µl)	Standard (µl)	Sample (µl)
Distilled H_2O	10µl	-----	--------
Standard	----	10µl	--------
Sample	-----	------	10µl
Reagent	1000µl	1000µl	1000µl

The solution was mixed and incubated for 5 minutes at 37^0c. The absorbance of the sample (A_{sample}) was measured against the reagent blank within 60 minutes.

Calculation:

Conc. Of Cholesterol in sample

$$= \frac{\Delta\Delta A_{sample}}{A_{standard}} \text{ x Conc. of standard (mg/dl)}$$

Determination of Plasma HDL-Cholesterol (Trinder, 1969; Tietz, 1990).

Assay Principle

Low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL (high density lipoprotein) fraction, which remains in the supernatant, is determined (Tietz, 1990).

Procedure:

1. **Precipitation**

The following were pipetted into centrifuge tubes;

Sample/standard	500µl
Diluted precipitant (R1)	1000µl

The solution was mixed and allowed to sit for 10 minutes at room temperature, after which it was centrifuged for 10 minutes at 4,000rpm. The clear supernatant was separated off within two hours and the cholesterol content was determined by the CHOD-PAP method.

2. **Cholesterol CHOD-PAP Assay**

The following were pipetted into test tube;

	Reagent blank	Standard	Sample
Distilled H_20	100µl	------	------
Standard supernatant	------	100µl	-------
Sample supernatant	-------	-------	100µl
CHOL reagent	1000µl	1000µl	1000µl

The solutions are mixed for 5 minutes at 37^0c. The absorbance of the sample (A_{sample}) and standard ($A_{standard)}$ was measured against the reagent blank within 60 minutes at a wavelength of 500nm.

Calculation:

1. HDL Cholesterol

Concentration of HDL cholesterol in supernatant

$$= \frac{\Delta A_{sample}}{\Delta A_{standard}} \times \text{Concentration of Standard}$$

Determination of Plasma Triglycerides (Trinder, 1969; Tietz, 1990).

Principle:

The triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase

Triglycerides + H_2O $\xrightarrow{\text{-lipase}}$ glycerol + fatty acids

Glycerol + ATP $\xrightarrow{GK}$ glycerol -3- phosphate + ADP

Glycerol -3- phosphate + 0_2 $\xrightarrow{GPO}$ dihydroxyacetone + phosphate + H_2O_2

$2H_2O_2$ + 4 – aminophenazone + 4 chlorophenol $\xrightarrow{POD}$ quinoneimine + HCL + $4H_2O$

Note: to correct for free glycerol, subtract 0.11mmol/l (10mg/dl) from the triglyceride value obtained.

Procedure:

The following were pipetted into test tubes:

	Reagent blank	Standard	Sample
Standard	--------	10µl	------
Sample	--------	------	10µl
Reagent R1	1000µl	1000µl	1000µl

Solution was mixed and incubated for 5 minutes at 37^0c, and the absorbance of the sample (A_{sample}) and standard ($A_{standard}$) was measured against the reagent blank within 60 minutes at wavelength of 500nm/546nm.

Calculation:

Triglyceride concentration= $\frac{A_{sample}}{A_{standard}}$ x Standard Conc.
(mmol/l or mg/dl)

Determination of serum LDL-cholesterol (Trinder, 1969; Tietz, 1990).

LDL-cholesterol is calculated using the Frieldewald equation as follows:

In mmol/l:

LDL Cholesterol = Total Cholesterol - $\frac{\text{Triglycerides}}{2.2}$ - HDL Cholesterol

In mg/dl:

LDL = Total - $\frac{\text{Triglycerides}}{}$ - HDL

Cholesterol	Cholesterol	5	Cholesterol

Determination of serum VLDL-cholesterol (Frieldewald *et al.,* 1972).

VLDL-cholesterol will be calculated using the Frieldewald's formula:

In mmol/l:

$$\text{VLDL- cholesterol} = \frac{\text{Triglycerides}}{2.2}$$

In mg/dl:

$$\text{VLDL- cholesterol} = \frac{\text{Triglycerides}}{5}$$

CHAPTER FOUR

4.0 RESULTS

The results shown in table 4.1 reveals that there was a statistically significant difference ($p<0.05$) in food and water intake across all the groups (group 1,2,3,4).

There was statistically significant difference ($p<0.05$) in weight gained when control (Group 1) was compared to the negative control (Group 2) and treatment groups; Group 3 (Atorvastatin) and Group 4 (High dose Yoyo cleanser bitters).

There was no statistically significant difference ($p>0.05$) when group 2 was compared with treatment groups; Group 3 (Atorvastatin) and Group 4 Group 3 (Atorvastatin) and Group 4 (High dose Yoyo cleanser bitters).

However there was statistically significant decrease ($p<0.05$) in weight gained when compared to control (Group 1).

There was a statistically significant difference ($p<0.05$) in feed efficiency when control (Group 1) was compared to Group 2 (Negative control) and Group 4 (High dose Yoyo cleanser bitters).

However there was no significant difference ($p>0.05$) when compared with Group 4

Table 4.1 Showing Food Consumed/day/rat(g), Water Consumed/day/rat(ml), Weight Gained/day/rat(g), Feed Efficiency (%)

Groups	FOOD CONSUMED/ DAY/RAT (g)	WATER CONSUMED /DAY/RAT(ml)	WEIGHT GAINED/DAY/RAT(g)	FEED EFFICIENCY(%)
Group 1 (Control)	16.73±0.00[a]	51.63±0.00[a]	0.67±0.09[a]	4.00±0.54[a]
Group 2 (HFD+FW)	8.61±0.00[b,c]	47.00±0.00[b,c]	-2.86±0.15[b,c]	-3.32±1.73[b,c]
Group 3 (HFD+FW + Atorvastatin)	10.34±0.00[b,d,e]	54.13±0.00[b,d,e]	-0.30±0.22[b,c,d]	-2.92±2.10[b,c,d]
Group 4 (HFD+FW + HD Yoyo bitters)	9.15±0.00[b,d,f]	48.54±0.00[b,d,f]	-0.14±0.09[b,c,d]	-1.56±0.98[a,c,d]

All research results are expressed as mean ± SEM (= standard error of the mean) of five determinations (n=5) and subjected to one-way analysis of variance (ANOVA). Means in the same column with different superscript alphabets on the same position, differ significantly at 95% level of significance ($p<0.05$) using Turkey's test of significance. HFD+FW: High fructose diet + 20% fructose water, HD Yoyo: High Dose Yoyo Cleanser bitters

The results in table 4.2 shows changes in lipid profile parameters of High dose Yoyo bitters treatment on *Wistar* rats fed a high fructose diet. After 56 days of experimentation, there was a significant increase ($p<0.05$) in Total Cholesterol levels in Group 2 compared to the control group (Group 1). Additionally, there was a significant increase ($p<0.05$) when compared to the treatment groups; Group 3 (Atorvastatin) and Group 4 (High dose Yoyo cleanser bitters). Also there was a significant increase ($p<0.05$) in Group 3 when compared to control group (Group 1).

However there was no significant difference ($p>0.05$) in total cholesterol levels between treatment groups; Group 3 (Atorvastatin) and Group 4 (High dose Yoyo cleanser bitters) when compared.

Triglyceride levels in Group 2 showed a significant increase ($p<0.05$) compared to both the control group and the treatment groups; Group 3 (Atorvastatin) and Group 4 (High dose Yoyo cleanser bitters).

A significant difference ($p<0.05$) in triglyceride levels was also observed between Group 3 and Group 4, with Group 4 (High dose Yoyo cleanser bitters) having notably lower triglyceride levels than Group 3 (Atorvastatin).

However, there was no significant difference ($p>0.05$) between the control group (Group 1) and the treatment groups; Group 3 (Atorvastatin) and Group 4 (High dose Yoyo cleanser bitters).

High-density lipoprotein cholesterol (HDL) levels in Group 2 significantly decreased ($p<0.05$) compared to both the control group (Group 1) and the treatment groups; Group 3 (Atorvastatin) and Group 4 (High dose Yoyo cleanser bitters).

Additionally, there was significant decrease ($p<0.05$) in control group (Group 1) when compared with treatment groups; Group 3 (Atorvastatin) and Group 4 (High dose Yoyo cleanser bitters).

However, there was no significant difference ($p>0.05$) between Group 3 (Atorvastatin) and Group 4 (High dose Yoyo cleanser bitters) when compared with each other.

Low density lipoprotein cholesterol levels were observed to have significantly increased ($p<0.05$) in Group 2 when compared with the control group and also in comparison with the other treatment groups; Group 3 (Atorvastatin) and Group 4 (High dose Yoyo cleanser bitters).

However, there was no significant difference ($p>0.05$) between the treatment groups ; Group 3 (Atorvastatin) and Group 4 (High dose Yoyo cleanser bitters)

and the control group (Group 1).

Very low-density lipoprotein cholesterol showed significant increase ($p<0.05$) in Group 2 when compared with the control group (Group 1) and the treatment groups; Group 3 (Atorvastatin) and Group 4 (High dose Yoyo cleanser bitters).

Also, there was a significant difference ($p<0.05$) between Group 3 (Atorvastatin) and Group 4 (High dose Yoyo cleanser bitters) when compared with each other.

Table 4.2: Lipid profile indices of the rats fed high fructose diet and 20% fructose water, atorvastatin and High Dose Yoyo bitters

Groups	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL(mg/dl)	LDL(mg/dl)	VLDL(mg/dl)
Control	67.18±2.81[a]	59.54±2.44[a]	29.20±2.67[a]	26.06±3.66[a]	11.92±0.49[a]
HFD+FW	94.44±1.56[b,c]	99.08±3.56[b,c]	20.60±1.72[b,c]	54.04±3.12[b,c]	19.80±0.32[b,c]
HFD+FW + Atorvastatin	74.50±0.75[b,d,e]	64.92±2.42[a,d,e]	45.40±2.06[b,d,e]	16.10±2.58[a,d,e]	13.00±0.48[a,d,e]
HFD+FW + HD Yoyo bitters	73.08±0.74[a,d,,e]	53.26±1.40[a,d,f]	43.40±1.21[b,d,e]	19.00±1.88[a,d,e]	10.68±0.28[a,d,f]

All research results are expressed as mean ± SEM (= standard error of the mean) of five determinations (n=5) and subjected to one-way analysis of variance (ANOVA). Experimental groups along each row designated with different letters differ significantly ($p<0.05$), using Turkey's test of significance. HFD+FW: High fructose diet + 20% fructose water, HD Yoyo: High Dose Yoyo Cleanser bitters

CHAPTER FIVE

5.0 DISCUSSION

The experimental results discussed in the previous chapter offer valuable insights into the effects of a high fructose diet on various lipid parameters and the potential therapeutic interventions to counteract these effects. The study's findings enhance our understanding of how high fructose consumption impacts lipid metabolism and highlight the potential role of both pharmaceutical and herbal interventions in managing these effects.

The study includes comparisons between a normal control group, a negative control group (rats fed high fructose diet and fructose water), a positive control group (rats fed high fructose diet + fructose water and Atorvastatin) and the experimental group (high fructose diet + fructose water and Yoyo bitters).

This study's evaluation of the lipid profile revealed elevated levels of triglycerides, total cholesterol, LDL, and VLDL in the negative control group (high fructose diet fed wistar rats) was noticeably higher than the normal control. This is in line with the study of (Haidari et al., 2002) which state that a substantial portion of dietary fructose is transformed into lipids through de novo synthesis of fatty acids an atherogenic lipid profile.

Furthermore, Atorvastatin led to a decrease in triglycerides, total cholesterol, LDL, VLDL levels showcasing the lipid-lowering ability of statins (McIver and Siddique, 2024).

Yoyo cleanser bitters exhibit hypocholesterolemic and hypotriacylglycerolemic effects, reducing LDL-cholesterol (bad cholesterol) and VLDL-cholesterol levels while increasing HDL-cholesterol (good cholesterol) levels.This effectiveness can be

attributed to one of its constituents known as flavonoid that showcase hypocholesterolemic and hypotriacylglycerolemic in the blood (Anionye *et al.,* 2017). Comparative Potency: Yoyo cleanser bitters demonstrated greater potency than Atorvastatin in reducing total cholesterol, triglycerides, low-density lipoprotein, very low-density lipoprotein, while increasing high-density lipoprotein levels. This suggests that Yoyo cleanser bitter may be a superior alternative to Atorvastatin for protecting against elevated lipid profile parameters, such as total cholesterol, triglycerides, low-density lipoprotein, and very low-density lipoprotein, induced by high fructose intake.

CONCLUSION

The findings of this study indicate that both high-dose Yoyo cleanser bitters and Atorvastatin show potential in mitigating alterations caused by a high fructose diet. Yoyo cleanser bitters, in particular, displayed promising results, even outperforming Atorvastatin in certain aspects of lipid profile regulation. Further research is necessary to uncover the mechanisms behind these effects and to investigate the clinical implications for individuals vulnerable to changes in lipid profile levels due to high fructose intake. These findings offer hope for the development of supplementary interventions to manage dyslipidemia.

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APPENDIX

I: DOSAGE REGIMEN FOR TREATMENTS

I. Dosage Regimen for Yoyo Cleanser Bitters

If 80ml is consumed by a 70,000g man (70kg)

How many ml will a 220g rat be expected to consume?

$$X\ ml = \frac{80ml \times 220g}{70{,}000g} = 0.251ml \text{ (approximately 0.25ml)}$$

0.25ml for a 220g rat means a dose of 0.25ml/220g = approx. 1.1 x10-3ml/g of rat (equivalent to: 1.1ml/kg of rat or 1.1g/kg or 1100 mg/kg).

Similar calculations will be done for distilled water. The rats will be weighed weekly and the weight used to calculate the equivalent doses/volume to be administered for each group of rats for the week.

II. Dosage Regimen for Atorvastatin

40mg is consumed by a 70,000g man (70kg)

A 220g rat was then expected to consume (X mg)

$$X\ mg = \frac{40mg \times 220g}{70{,}000g} = 0.126mg$$ (which meant dissolving 0.126mg of the drug in 1ml of distilled water)

0.126mg for a 220g rat means a dose of 0.126mg/220g = approx. 0.57x10-3mg/g of rat. (0.57mg/kg of rat body weight).

II: RAW DATA

FEED CONSUMED/DAY/RAT (g) OF MALE WISTAR RAT FED A HIGH FRUCTOSE DIET ANALYSIS

Descriptives

Feed_Consumed

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	5	16.7300	.00000	.00000	16.7300	16.7300	16.73	16.73
2	5	8.6100	.00000	.00000	8.6100	8.6100	8.61	8.61
3	5	10.3400	.00000	.00000	10.3400	10.3400	10.34	10.34
4	5	9.1500	.00000	.00000	9.1500	9.1500	9.15	9.15
Total	20	10.9800	3.00229	.60046	9.7407	12.2193	8.61	16.73

ANOVA

Feed_Consumed

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	216.330	4	54.083	.	.
Within Groups	.000	20	.000		
Total	216.330	24			

Multiple Comparisons

Dependent Variable: feed_consumed

Tukey HSD

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	8.12000*	.00211	.000	8.1131	8.1269
	3	6.39000*	.00211	.000	6.3831	6.3969
	4	7.57667*	.00211	.000	7.5697	7.5836
2	1	-8.12000*	.00211	.000	-8.1269	-8.1131
	3	-1.73000*	.00211	.000	-1.7369	-1.7231
	4	-.54333*	.00211	.000	-.5503	-.5364
3	1	-6.39000*	.00211	.000	-6.3969	-6.3831
	2	1.73000*	.00211	.000	1.7231	1.7369
	4	1.18667*	.00211	.000	1.1797	1.1936
4	1	-7.57667*	.00211	.000	-7.5836	-7.5697
	2	.54333*	.00211	.000	.5364	.5503

	3	-1.18667*	.00211	.000	-1.1936	-1.1797

*. The mean difference is significant at the 0.05 level.

WATER CONSUMED/DAY/RAT (ml) OF MALE WISTAR RAT FED A HIGH FRUCTOSE DIET

Descriptives

Water_Consumed

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	5	51.6300	.00000	.00000	51.6300	51.6300	51.63	51.63
2	5	47.0000	.00000	.00000	47.0000	47.0000	47.00	47.00
3	5	54.1300	.00000	.00000	54.1300	54.1300	54.13	54.13
4	5	48.5400	.00000	.00000	48.5400	48.5400	48.54	48.54
Total	20	50.3520	2.51816	.50363	49.3126	51.3914	47.00	54.13

ANOVA

Water_Consumed

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	152.187	4	38.047	.	.
Within Groups	.000	20	.000		
Total	152.187	24			

Multiple Comparisons

Dependent Variable: water_consumed

Tukey HSD

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	4.63000*	.00126	.000	4.6262	4.6338
	3	-2.50200*	.00126	.000	-2.5058	-2.4982
	4	3.09000*	.00126	.000	3.0862	3.0938
2	1	-4.63000*	.00126	.000	-4.6338	-4.6262
	3	-7.13200*	.00126	.000	-7.1358	-7.1282
	4	-1.54000*	.00126	.000	-1.5438	-1.5362
3	1	2.50200*	.00126	.000	2.4982	2.5058
	2	7.13200*	.00126	.000	7.1282	7.1358
	4	5.59200*	.00126	.000	5.5882	5.5958

4	1	-3.09000*	.00126	.000	-3.0938	-3.0862
	2	1.54000*	.00126	.000	1.5362	1.5438
	3	-5.59200*	.00126	.000	-5.5958	-5.5882

*. The mean difference is significant at the 0.05 level.

WEIGHT GAINED PER DAY PER RAT(g)

Descriptives

Weight_gained_per_day_per_rat

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	5	.6700	.20518	.09176	.4152	.9248	.43	.96
2	5	-.2860	.33389	.14932	-.7006	.1286	-.74	.07
3	5	-.3000	.48785	.21817	-.9057	.3057	-.85	.49
4	5	-.1440	.20107	.08992	-.3937	.1057	-.35	.17
Total	20	-.0800	.47864	.09573	-.2776	.1176	-.85	.96

ANOVA

Weight_gained_per_day_per_rat

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.625	4	.906	9.676	.000
Within Groups	1.873	20	.094		
Total	5.498	24			

Multiple Comparisons

Dependent Variable: Weight_gained_per_day_per_rat

	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1	2	.95600*	.19356	.001	.3768	1.5352
		3	.97000*	.19356	.001	.3908	1.5492
		4	.81400*	.19356	.004	.2348	1.3932
	2	1	-.95600*	.19356	.001	-1.5352	-.3768
		3	.01400	.19356	1.000	-.5652	.5932
		4	-.14200	.19356	.946	-.7212	.4372
	3	1	-.97000*	.19356	.001	-1.5492	-.3908
		2	-.01400	.19356	1.000	-.5932	.5652

		4	-.15600	.19356	.926	-.7352	.4232
	4	1	-.81400*	.19356	.004	-1.3932	-.2348
		2	.14200	.19356	.946	-.4372	.7212
		3	.15600	.19356	.926	-.4232	.7352

*. The mean difference is significant at the 0.05 level.

FEED EFFICIENCY (%) OF MALE WISTAR RAT FED A HIGH FRUCTOSE DIET

Descriptives

Feed_Efficiency

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	5	4.00	1.217	.544	2.49	5.51	3	6
2	5	-3.32	3.870	1.731	-8.13	1.49	-9	1
3	5	-2.92	4.701	2.102	-8.76	2.92	-8	5
4	5	-1.56	2.201	.984	-4.29	1.17	-4	2
Total	20	-1.44	4.000	.800	-3.09	.21	-9	6

ANOVA

Feed_Efficiency

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	195.872	4	48.968	5.206	.005
Within Groups	188.108	20	9.405		
Total	383.980	24			

Multiple Comparisons

Dependent Variable: Feed_Efficiency

	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1	2	7.320*	1.940	.009	1.52	13.12
		3	6.920*	1.940	.015	1.12	12.72
		4	5.560	1.940	.064	-.24	11.36
	2	1	-7.320*	1.940	.009	-13.12	-1.52
		3	-.400	1.940	1.000	-6.20	5.40

		4	-1.760	1.940	.891	-7.56	4.04
	3	1	-6.920*	1.940	.015	-12.72	-1.12
		2	.400	1.940	1.000	-5.40	6.20
		4	-1.360	1.940	.954	-7.16	4.44
	4	1	-5.560	1.940	.064	-11.36	.24
		2	1.760	1.940	.891	-4.04	7.56
		3	1.360	1.940	.954	-4.44	7.16

*. The mean difference is significant at the 0.05 level.

LIPID PROFILE OF MALE WISTAR RATS FED A HIGH FRUCTOSE DIET ANALYSIS

1. Total Cholesterol

Descriptives

Total_Cholesterol

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	5	67.1800	6.27471	2.80614	59.3889	74.9711	60.10	73.20
2	5	94.4400	3.47606	1.55454	90.1239	98.7561	90.50	99.70
3	5	74.5000	1.66883	.74632	72.4279	76.5721	71.90	75.90
4	5	73.0800	1.65136	.73851	71.0296	75.1304	71.30	75.60
Total	20	76.3440	10.12123	2.02425	72.1662	80.5218	60.10	99.70

ANOVA

Total_Cholesterol

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2200.606	4	550.151	42.658	.000
Within Groups	257.936	20	12.897		
Total	2458.542	24			

Multiple Comparisons

Dependent Variable: Total_Cholesterol

Tukey HSD

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-27.26000*	2.27128	.000	-34.0565	-20.4635
	3	-7.32000*	2.27128	.031	-14.1165	-.5235
	4	-5.90000	2.27128	.109	-12.6965	.8965
2	1	27.26000*	2.27128	.000	20.4635	34.0565
	3	19.94000*	2.27128	.000	13.1435	26.7365
	4	21.36000*	2.27128	.000	14.5635	28.1565
3	1	7.32000*	2.27128	.031	.5235	14.1165
	2	-19.94000*	2.27128	.000	-26.7365	-13.1435
	4	1.42000	2.27128	.969	-5.3765	8.2165
4	1	5.90000	2.27128	.109	-.8965	12.6965
	2	-21.36000*	2.27128	.000	-28.1565	-14.5635
	3	-1.42000	2.27128	.969	-8.2165	5.3765

*. The mean difference is significant at the 0.05 level.

2. Triglyeride

Descriptives

Triglyceride

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	5	59.5400	5.44546	2.43528	52.7786	66.3014	50.50	65.30
2	5	99.0800	3.56258	1.59324	94.6565	103.5035	95.70	105.10
3	5	64.9200	5.40990	2.41938	58.2027	71.6373	59.80	70.90
4	5	53.2600	3.12538	1.39771	49.3793	57.1407	50.80	58.40
Total	20	67.4320	17.00938	3.40188	60.4109	74.4531	50.50	105.10

ANOVA

Triglyceride

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6605.242	4	1651.311	97.592	.000
Within Groups	338.412	20	16.921		
Total	6943.654	24			

Multiple Comparisons

Dependent Variable: Triglyceride

Tukey HSD

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-39.54000*	2.60158	.000	-47.3249	-31.7551
	3	-5.38000	2.60158	.272	-13.1649	2.4049
	4	6.28000	2.60158	.152	-1.5049	14.0649
2	1	39.54000*	2.60158	.000	31.7551	47.3249
	3	34.16000*	2.60158	.000	26.3751	41.9449
	4	45.82000*	2.60158	.000	38.0351	53.6049
3	1	5.38000	2.60158	.272	-2.4049	13.1649
	2	-34.16000*	2.60158	.000	-41.9449	-26.3751
	4	11.66000*	2.60158	.002	3.8751	19.4449
4	1	-6.28000	2.60158	.152	-14.0649	1.5049
	2	-45.82000*	2.60158	.000	-53.6049	-38.0351
	3	-11.66000*	2.60158	.002	-19.4449	-3.8751

*. The mean difference is significant at the 0.05 level.

3. HDL

Descriptives

HDL

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	5	29.2000	5.97495	2.67208	21.7811	36.6189	20.00	35.00
2	5	20.6000	3.84708	1.72047	15.8232	25.3768	16.00	26.00
3	5	45.4000	4.61519	2.06398	39.6695	51.1305	40.00	50.00
4	5	43.4000	2.70185	1.20830	40.0452	46.7548	40.00	47.00
Total	20	36.8000	11.03026	2.20605	32.2469	41.3531	16.00	50.00

ANOVA

HDL

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2558.400	4	639.600	35.376	.000
Within Groups	361.600	20	18.080		
Total	2920.000	24			

Multiple Comparisons

Dependent Variable: HDL

Tukey HSD

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	8.60000*	2.68924	.033	.5528	16.6472
	3	-16.20000*	2.68924	.000	-24.2472	-8.1528
	4	-14.20000*	2.68924	.000	-22.2472	-6.1528
2	1	-8.60000*	2.68924	.033	-16.6472	-.5528
	3	-24.80000*	2.68924	.000	-32.8472	-16.7528
	4	-22.80000*	2.68924	.000	-30.8472	-14.7528
3	1	16.20000*	2.68924	.000	8.1528	24.2472
	2	24.80000*	2.68924	.000	16.7528	32.8472
	4	2.00000	2.68924	.943	-6.0472	10.0472
4	1	14.20000*	2.68924	.000	6.1528	22.2472
	2	22.80000*	2.68924	.000	14.7528	30.8472
	3	-2.00000	2.68924	.943	-10.0472	6.0472

*. The mean difference is significant at the 0.05 level.

4. LDL

Descriptives

LDL

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	5	26.0600	8.18706	3.66137	15.8944	36.2256	19.90	39.90
2	5	54.0400	6.98019	3.12163	45.3730	62.7070	44.80	62.20
3	5	16.1000	5.76628	2.57876	8.9402	23.2598	9.70	23.40
4	5	19.0000	4.21248	1.88388	13.7695	24.2305	12.60	23.40
Total	20	26.2920	15.59813	3.11963	19.8534	32.7306	9.70	62.20

ANOVA

LDL

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	5138.482	4	1284.621	36.664	.000

Within Groups	700.756	20	35.038		
Total	5839.238	24			

Multiple Comparisons

Dependent Variable: LDL

Tukey HSD

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-27.98000*	3.74368	.000	-39.1825	-16.7775
	3	9.96000	3.74368	.097	-1.2425	21.1625
	4	7.06000	3.74368	.356	-4.1425	18.2625
2	1	27.98000*	3.74368	.000	16.7775	39.1825
	3	37.94000*	3.74368	.000	26.7375	49.1425
	4	35.04000*	3.74368	.000	23.8375	46.2425
3	1	-9.96000	3.74368	.097	-21.1625	1.2425
	2	-37.94000*	3.74368	.000	-49.1425	-26.7375
	4	-2.90000	3.74368	.935	-14.1025	8.3025
4	1	-7.06000	3.74368	.356	-18.2625	4.1425
	2	-35.04000*	3.74368	.000	-46.2425	-23.8375
	3	2.90000	3.74368	.935	-8.3025	14.1025

*. The mean difference is significant at the 0.05 level.

5. VLDL

Descriptives

VLDL

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	5	11.9200	1.10091	.49234	10.5530	13.2870	10.10	13.10
2	5	19.8000	.71414	.31937	18.9133	20.6867	19.10	21.00
3	5	13.0000	1.06536	.47645	11.6772	14.3228	12.00	14.20
4	5	10.6800	.62209	.27821	9.9076	11.4524	10.20	11.70
Total	20	13.4960	3.38840	.67768	12.0973	14.8947	10.10	21.00

ANOVA

VLDL

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	262.026	4	65.506	96.874	.000
Within Groups	13.524	20	.676		
Total	275.550	24			

Multiple Comparisons

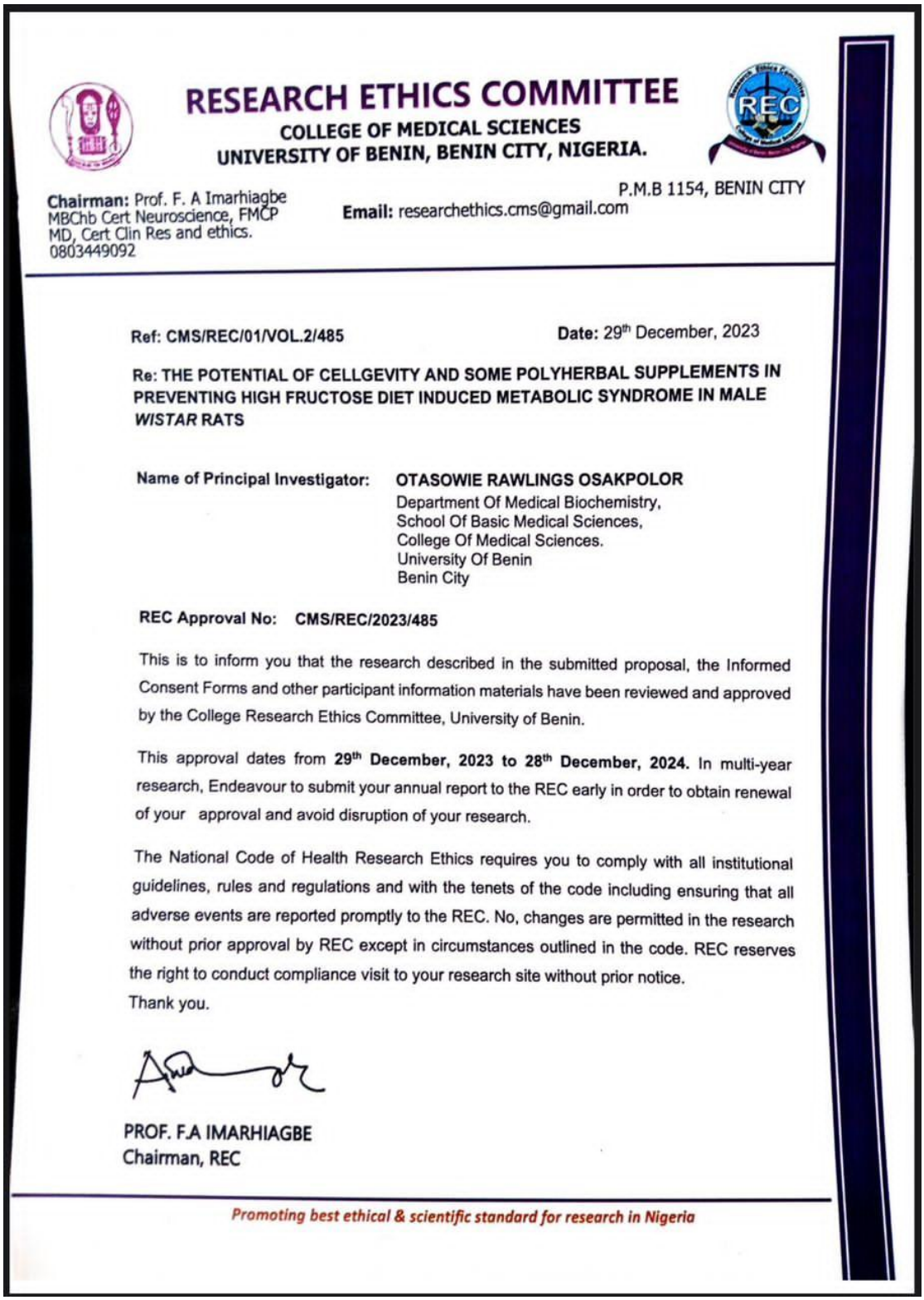
Dependent Variable: VLDL

Tukey HSD

		Mean Difference			95% Confidence Interval	
(I) Groups	(J) Groups	(I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
1	2	-7.88000*	.52008	.000	-9.4363	-6.3237
	3	-1.08000	.52008	.268	-2.6363	.4763
	4	1.24000	.52008	.161	-.3163	2.7963
2	1	7.88000*	.52008	.000	6.3237	9.4363
	3	6.80000*	.52008	.000	5.2437	8.3563
	4	9.12000*	.52008	.000	7.5637	10.6763
3	1	1.08000	.52008	.268	-.4763	2.6363
	2	-6.80000*	.52008	.000	-8.3563	-5.2437
	4	2.32000*	.52008	.002	.7637	3.8763
4	1	-1.24000	.52008	.161	-2.7963	.3163
	2	-9.12000*	.52008	.000	-10.6763	-7.5637
	3	-2.32000*	.52008	.002	-3.8763	-.7637

*. The mean difference is significant at the 0.05 level.

III: ETHICAL CLEARANCE



RESEARCH ETHICS COMMITTEE
COLLEGE OF MEDICAL SCIENCES
UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

Chairman: Prof. F. A Imarhiagbe
MBChb Cert Neuroscience, FMCP
MD, Cert Clin Res and ethics.
0803449092

P.M.B 1154, BENIN CITY
Email: researchethics.cms@gmail.com

Ref: CMS/REC/01/VOL.2/485 **Date:** 29th December, 2023

Re: THE POTENTIAL OF CELLGEVITY AND SOME POLYHERBAL SUPPLEMENTS IN PREVENTING HIGH FRUCTOSE DIET INDUCED METABOLIC SYNDROME IN MALE *WISTAR* RATS

Name of Principal Investigator: **OTASOWIE RAWLINGS OSAKPOLOR**
Department Of Medical Biochemistry,
School Of Basic Medical Sciences,
College Of Medical Sciences.
University Of Benin
Benin City

REC Approval No: CMS/REC/2023/485

This is to inform you that the research described in the submitted proposal, the Informed Consent Forms and other participant information materials have been reviewed and approved by the College Research Ethics Committee, University of Benin.

This approval dates from **29th December, 2023 to 28th December, 2024.** In multi-year research, Endeavour to submit your annual report to the REC early in order to obtain renewal of your approval and avoid disruption of your research.

The National Code of Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the code including ensuring that all adverse events are reported promptly to the REC. No, changes are permitted in the research without prior approval by REC except in circumstances outlined in the code. REC reserves the right to conduct compliance visit to your research site without prior notice.
Thank you.

PROF. F.A IMARHIAGBE
Chairman, REC

Promoting best ethical & scientific standard for research in Nigeria