THE COMPARATIVE EFFECT OF CO-ADMINISTRATION OF LOSARTAN/METFORMIN OR (AND) LOSARTAN/GLIBENCLAMIDE ON PLASMA LIPID PROFILE OF L-NAME/STREPTOZOTOCIN INDUCED HYPERTENSIVE/DIABETIC MALE WISTAR RAT

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ABSTRACT

Hypertension and diabetes mellitus frequently coexist, significantly increasing cardiovascular disease risk due to dyslipidemia, oxidative stress, and endothelial dysfunction. This study investigates the impact of co-administering losartan/metformin (L/M) and losartan/glibenclamide (L/D) on plasma and cardiac lipid profiles in L-NAME/streptozotocin (STZ)-induced hypertensive/diabetic male Wistar rats, with a focus on total cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol. Fifty-two male Wistar rats (50–80 g) were housed under standard laboratory conditions and acclimatized for two weeks. Hypertension was induced by administering L-NAME (40 mg/kg) in drinking water for four weeks, while diabetes was induced via a single intraperitoneal injection of STZ (50 mg/kg). Successful induction was confirmed by systolic blood pressure measurement (tail-cuff method) and fasting blood glucose levels >200 mg/dL. The rats were divided into six groups (n=6 per group): control, untreated, diabetic treated with L/M, and diabetic treated with L/D. Drug administration was conducted orally for 28 days. At the end of the treatment period, rats were sacrificed via cervical dislocation. Blood samples were collected via cardiac puncture into EDTA-coated tubes, centrifuged at 3,000 rpm for 10 minutes, and plasma was stored at −20°C for lipid profile analysis. Cardiac tissues were excised, homogenized in phosphate-buffered saline (PBS), and centrifuged to obtain supernatants for further analysis. Plasma and cardiac total cholesterol levels did not significantly differ between groups ($p > 0.05$). The hypertensive/diabetic group exhibited reduced HDL-cholesterol (85.0 ± 19.2 mg/dL) compared to the normotensive/non-diabetic control (110.8 ± 2.1 mg/dL). Treatment with L/D showed the highest HDL levels (2.87 ± 2.71 mg/dL), suggesting a possible beneficial effect of glibenclamide on HDL metabolism. Triglyceride levels varied significantly ($p < 0.05$), with hypertensive/diabetic rats showing elevated plasma (160.0 ± 27.2 mg/dL) and cardiac (27.92 ± 12.55 mg/dL) triglycerides compared to controls. L/M treatment reduced plasma triglycerides to 145.2 ± 17.7 mg/dL, while L/D increased them to 180.3 ± 51.0 mg/dL, suggesting metformin's superior role in mitigating hypertriglyceridemia. Plasma LDL-cholesterol levels remained unchanged across all groups ($p > 0.05$), indicating limited effects of these drug regimens on LDL metabolism. This study highlights the differential impact of losartan/metformin and losartan/glibenclamide co-administration on lipid

metabolism in hypertensive/diabetic rats. These findings contribute to optimizing therapeutic strategies for managing dyslipidemia in comorbid hypertension and diabetes.

CHAPTER ONE

1 INTRODUCTION AND LITERATURE REVIEW

1.1: INTRODUCTION

Hypertension and diabetes are among the most prevalent chronic diseases worldwide, and their co-morbidity represents a critical concern in modern public health. Hypertension is another name for high blood pressure. It is a chronic medical condition in which the blood pressure in the arteries is raised (Marshall *et al.,* 2020). The higher the pressure in the blood vessels the harder the heart has to work in order to pump blood.

Diabetes is a chronic disease that causes high levels of glucose in the blood. Both conditions are major contributors to cardiovascular disease, stroke, kidney failure, and other life-threatening complications, leading to high healthcare costs and a substantial burden on healthcare systems globally. The co-occurrence of hypertension and diabetes increases the risk of these adverse outcomes, amplifying their individual effects on morbidity and mortality. Given their high prevalence, the co-morbidity of hypertension and diabetes has become a focal point of clinical research and public health interventions (Ogah *et al*., 2021).

An estimated 1.13 billion people worldwide have hypertension, most living in low and middle income countries. In 2015, one in four men and one in five women had hypertension. Fewer people with hypertension have the problem under control making it a multiple risk factor for cardiovascular disease (Novo *et al.,* 2019). Diabetes is similarly widespread, affecting an estimated 537 million adults globally in 2021, a number projected to rise significantly in the coming decades (International Diabetes Federation, 2021).

The co-existence of hypertension and diabetes is common, with studies suggesting that up to 50% of people with diabetes also have high blood pressure (Egan et al., 2021). Conversely,

individuals with hypertension are more likely to develop insulin resistance and, eventually, diabetes. This shared epidemiological burden contributes to the higher incidence of cardiovascular events, kidney disease, and overall mortality in people with both conditions.

1.2: TYPES OF HYPERTENSION

There are two primary types of hypertension. For 95% of people with high blood pressure, the cause of hypertension is unknown- this is called essential or primary hypertension. When a cause can be found, the condition is called secondary hypertension.

1.2.1: PRIMARY HYPERTENSION

Doctors diagnose this hypertension type after analysing patient blood pressure after three or four visits (Franklin *et al*., 2019). People who suffer from this hypertension type show no significant symptoms, However, a few patients show the following signs: headaches, fatigue, dizziness or nosebleeds (Cain and Khalil, 2022).

Haynes and Webb (2020) stated that primary hypertension is seen approximately 95% of the time, ranking as the most commonly occurring form of hypertension and refers to elevated blood pressure from multiple abnormalities in the regulatory functions. These abnormalities may vary from dysfunction in calcium, potassium or sodium levels, vascular contractions, hormonal elevations and central nervous system stimulations.

1.2.2: SECONDARY HYPERTENSION

This hypertension type occurs when there is an abnormality in the arteries that supply blood to the kidneys. Some common causes of this hypertension includes: abnormalities or tumours of the adrenal glands, hormonal imbalances and alcohol intake (Howell *et al*., 2018). Secondary hypertension occurs when the patient has no family history of hypertension with no obvious reasons for a diagnosis. It refers to a discernible underlying cause of blood pressure elevation

that may include, but is not limited to renal artery stenosis, primary aldosteronism or hyperthyroidism (Williams *et al*.,2021). The most common causes of secondary hypertension is an abnormality in the arteries supplying blood to the kidneys. Other causes include airway obstrucution during sleep, diseases of the adrenal glands and too much salt in the diet. Drugs can cause secondary hypertension, including over-the-counter medications such as ibuprofen and pseudoephedrine. If a secondary condition is diagnosed and curable, then removing this cause is typically associated with marked improvement in hypertension (Weinberger, 2016).

1.2.3: MALIGNANT HYPERTENSION

Malignant hypertension is defined as extremely high blood pressure that leads to progressive damage to target organs. The key feature distinguishing malignant hypertension from other forms of severe hypertension is end-organ damage, which typically involves the retina (hypertensive retinopathy), the brain (encephalopathy or stroke), and the kidneys (renal failure). The term "malignant" refers to the rapid progression of the disease, which can cause irreversible damage within a matter of days to weeks if not treated promptly (Hansson *et al*., 2013).

1.2.4: RESISTANT HYPERTENSION

Resistant hypertension (RH) is defined as hypertension that remains uncontrolled despite the use of at least three antihypertensive medications, including a diuretic, at optimal doses, and with adequate patient adherence. This type of hypertension is usually observed in people who are aged, obese or are suffering from diabetes or kidney ailments. It may occur in 20 - 30% of high blood pressure cases (Yusuf *et al*., 2017).

1.3: STAGES OF HYPERTENSION

A blood pressure between 120 and 129 mmHg for the top (systolic) number and more than 80mmHg (diastolic) for the bottom number is considered elevated. Blood pressure measurements are categorized as follows:

Normal: systolic less than 120mmHg and diastolic less than 80mmHg. No treatment is necessary but you should monitor your blood pressure to be sure that it remains within the normal range (Bundy *et al*., 2018).

Pre-hypertension/elevated: systolic between 120 - 129 mmHg and diastolic less than 80mmHg. This is just before the patient crosses the threshold for the development of hypertension but is at risk off developing hypertension. There is little or no evidence that using medications at this range is useful for preventing heart disease or stroke. People in this group are recommended life style measures to try to prevent the onset of hypertension. Lifestyle measures include exercise, managing body weight into a normal range, eating a diet high in fruits and vegetables, and choosing low-fat dairy products (Bell *et al*., 2018).

Stage 1: systolic between 130 - 139 mmHg or diastolic between 80 - 89 mmHg. Management includes the same lifestyle measures as with pre-hypertension and the use of a number of drugs known not only to reduce blood pressure but also to reduce the risk of heart disease and stroke. Classes of drugs include: thiazide diuretics, ACE inhibitors, angiotensin receptor blockers and calcium channel blockers. (Bundy *et al*., 2018).

Stage 2: systolic at least 140mmHg or diastolic at least 90mmHg. In addition to life style changes, patients are advised to choose a two-drug therapy from among the five classes of hypertensive agents used to get their blood pressure down (Adeloye *et al*., 2018).

1.4 PLASMA SODIUM ASSAY

Sodium is one of the most important electrolytes along with chlorine, calcium and potassium. Sodium plays vital roles in maintaining normal cell functions such as plasma volume, pH balance or transmission of nerve impulses. Healthy individuals can absorb sodium ingested in food, and kidneys maintain proper sodium balance by excreting its excess in urine. Normal sodium intake has been defined to be between 200-250 mg/day (Cordeiro, R.M. *et al*., 2023).

Hyponatremia (low sodium concentration in blood) can occur in patients with nephritic syndrome, excessive vomiting and diarrhoea, while Hypernatremia (high sodium concentration in blood) is developed in patients suffering from liver diseases, burns and pregnancy. Traditionally, sodium concentration in clinical settings is determined by potentiometric, gravimetry, photometry, titrimetry and flame atomic emission spectroscopy, but these methods require expensive and complex protocols that need to be performed by trained personnel.

Measurement of serum sodium is routine in assessing electrolyte, acid-base and water balance, as well as renal function. Sodium accounts for approximately 95% of the osmotically active substances in the extracellular compartment, provided that the patient is not in renal failure or does not have severe hyperglycemia.

1.4.1 PHYSIOLOGICAL ROLE OF SODIUM

Sodium is the most abundant cation in extracellular fluid and is responsible for the maintainance of the osmotic pressure of the ECF. Normally, it is present in serum in a concentration ranging from 135-155 mmol/l, according to Sussman, M.A. *et al*., (2022) and in a concentration of 140mmol/l. The main physiological function of sodium is the maintenance of the osmotic pressure in the fluid compartments of the body through regulation of plasma water volume. As part of the strong ions, sodium is involved in the regulation of the acid-base balance and

contributes to nerve and muscle functions through maintenance of membrane potentials and transmission of nerve impulses. In addition, sodium is involved in Na+/K+-ATPase, which is responsible for maintaining the electric gradients required for nutrient transport over membrane borders. Sodium also plays a role in the absorptive process of monosaccharides, amino acids, pyrimidines and bile salts (Thompson and Hoorn, 2019). Sodium constitutes a major component of saliva and together with bicarbonate serves to buffer acids that are generated during ruminal fermentation (Nogueira, R. *et al*., 2023).

1.4.2 SODIUM HOMEOSTASIS

Sodium is the main cation present in the ECF compartment with approximately 1/2 to 1/3 of body sodium in the form of available sodium. The remaining sodium is then bound in bone substances. The main absorption site of sodium is the GIT. Active and passive transport mechanisms are present in the reticulorumen, omasum , abomasum and the intestines (Titze, 2022). Normally, sodium is secreted in milk with 25 - 30mmol/l and increased losses occur in cows suffering from mastitis, due to the leakage of serum constituents into the milk (Rossignol, P. *et al*., 2023). The regulation of plasma sodium depends on the water balance. Since the available sodium content is the principal determinant of ECF volume, the sodium deficit is the principal cause of decreased ECF volume and increased sodium content then results in increased ECF volume (Titze, 2022).

The kidney plays a key role in the maintenance of ECF volume, as osmoreceptors and baroreceptors located in the kidney steer the sodium homeostasis. In the case of hypernatremia, osmoreceptors stimulate antidiuretic hormone (ADH) scretion from the pituitary gland and stimulate thirst, which then results in increased water intake and water retention by the kidney. To this end, increasing the water content therefore results in the lowering of the sodium

concentration and a subsequent return to normal. Opposite changes occur with hypo-osmolality. With the presence of hypovolemia (decreased ECF), baroreceptors stimulate the renin-angiotensin system, resulting in aldosterone release from the adrenal cortex. Hyperaldosteronism lead to absorption of sodium, chlorine and water (Rossignol, P. *et al*., 2023).

1.4.3: DISORDERS OF SODIUM BALANCE

Changes in serum sodium concentration depend on changes in the water balance. An abnormal increase in serum sodium level (less than 132 mmol/l) is termed hyponatremia while increase in sodium levels in serum (above 152mmol/l) characterize hypernatremia. Disturbancess of sodium balance affect the osmolarity and can result in fatality e.g in calves with neonatal diarrhoea (Byramji *et al*., 2018). Commonly, green forage does not contain sufficient quantities of sodium chloride as common salt to the diet or by allowing the animals to consume salt. In lactating dairy cows, the most common causes of chronic sodium depletion is feeding on a low salt diet and losses of sodium in the milk which is even increased in cows under stress or cows suffering from mastitis.

Normal results for this test are 135 - 145 mEq/L (milliequivalents per liter), according to (Ogah *et al*., 2020) but different laboratories use different values for “normal”. A blood sodium level lower than 135 mEq/L is called hyponatremis (Ogunniyi *et* al., 2021). Hyponatremia means high levels of sodium in the blood. It’s defined as levels that exceed 145 mEq/L.

1.5 DIABETES MELLITUS

Blood glucose dysregulation is a hallmark of diabetes mellitus, a set of metabolic diseases with serious long-term health consequences. Type 1 and type 2, the two main forms of diabetes, have different pathophysiological processes and clinical manifestations, necessitating specialised

therapeutic strategies. Effective management of diabetes and associated comorbidities requires an understanding of the subtle differences between these two illnesses

1.5.1 DIABETES TYPE 1

Insulin-producing beta cells in the pancreatic islets are selectively destroyed in type 1 diabetes, an autoimmune disease that was formerly classified as insulin-dependent or juvenile-onset diabetes (Atkinson *et al*., 2014). Because of this process, there is a complete lack of insulin, which makes it impossible to control blood glucose levels.

Type 1 diabetes has a complex aetiology that involves a complex interaction between environmental factors and genetic susceptibility. Numerous genetic loci, especially within the human leukocyte antigen (HLA) complex, have been linked to an elevated risk of type 1 diabetes by genome-wide association studies (Redondo *et al*., 2018). The immune system becomes dysregulated as a result of these genetic variables, preparing the body for an autoimmune reaction against its own beta cells in the pancreas.

Research on the specific environmental factors that set off the autoimmune response in type 1 diabetes is still ongoing. Atypical immunological responses and consequent beta cell death may be triggered by viral infections, especially those caused by enteroviruses (Hober and Sauter, 2010). Furthermore, in people who are genetically predisposed, dietary factors such early exposure to cow's milk proteins have been linked to an increased risk of developing type 1 diabetes (Knip *et al*., 2018).

Type 1 diabetes causes a progressive loss of beta cells that produce insulin, leaving the patient completely dependent on exogenous insulin therapy to survive. Diabetic ketoacidosis, a potentially fatal condition marked by the buildup of ketone bodies, frequently coexists with the hyperglycemia symptoms that patients with type 1 diabetes generally exhibit, such as increased

thirst, frequent urination, and weight loss (Wolfsdorf *et al*., 2018). Type 1 diabetes can cause serious side effects as diabetic nephropathy, retinopathy, neuropathy, and cardiovascular disease if insulin therapy is not started on time.

1.5.2 DIABETES TYPE 2

Insulin resistance and decreased insulin production interact intricately to cause type 2 diabetes, the most common kind of the disease. As opposed to type 1 diabetes, lifestyle variables such obesity, inactivity, and bad eating habits are usually linked to type 2 diabetes (Chatterjee *et al*., 2017). Insulin resistance is the main pathophysiological mechanism of type 2 diabetes, in which target tissues like skeletal muscle, liver, and adipose tissue lose their sensitivity to the effects of insulin. Pancreatic beta cells secrete more insulin in response to this decreased sensitivity to insulin, which impairs glucose absorption and utilisation (Kahn *et al*., 2014). The persistent need for more insulin production over time may cause beta cell malfunction and ultimately failure, which would make the hyperglycaemic condition worse. Type 2 diabetes is also significantly influenced by genetic factors. Numerous genetic variations, including those related to insulin production, insulin action, and the regulation of glucose homeostasis, have been linked to an increased risk of acquiring the illness by genome-wide association studies (Prasad and Groop, 2015). The total risk of type 2 diabetes is influenced by these genetic predispositions as well as environmental and lifestyle variables.

Compared to type 1 diabetes, type 2 diabetes frequently presents clinically more gradually and subtly. Patients may have little or no symptoms at first, and over time, hyperglycemia may develop. Increased thirst, frequent urination, exhaustion, and blurred vision are typical symptoms (American Diabetes Association, 2020). Type 2 diabetes can sometimes go years without a diagnosis, eventually being discovered during regular examinations or when complications arise.

1.6 LITERATURE REVIEW

One of the biggest problems facing modern medicine is the intricate link between diabetes and hypertension. According to recent epidemiological research, over 68% of individuals with diabetes go on to develop hypertension, and over time, diabetic symptoms develop in about 45% of patients with hypertension (Anderson *et al*., 2023). Recent studies have shed light on the pathophysiological mechanisms underpinning this link, exposing a complex network of vascular and metabolic changes that produce a self-reinforcing cycle of disease progression.

A key link in the connection between these disorders is endothelial dysfunction. According to research by Thompson *et al*., (2024), a persistent increase in blood pressure causes mechanical stress on the vascular endothelium, which raises the production of inflammatory mediators and reactive oxygen species (ROS). Endothelial cells' insulin signalling pathways are compromised by this oxidative stress environment, which lowers nitric oxide production and impairs vasodilation. The ensuing endothelial dysfunction feeds a vicious cycle of metabolic and vascular dysfunction by aggravating hypertension and encouraging insulin resistance in peripheral tissues.

The function of the renin-angiotensin-aldosterone system (RAAS) in this pathophysiological interaction has been emphasised by recent research. According to Martinez and associates (2023), hyperglycemia causes local RAAS in vascular tissues to become active, which raises angiotensin II production. In addition to encouraging vasoconstriction, this increase in angiotensin II also disrupts insulin-mediated glucose absorption in skeletal muscle, which exacerbates insulin resistance. According to the study, long-term RAAS activation causes vascular remodelling that is typified by an elevated media-to-lumen ratio and improved proliferation of vascular smooth muscle cells.

1.7 ***Simarouba glauca***

Simarouba glauca, (*medicinalis*) most commonly referred to as the "Paradise Tree" belongs to the family of Simaroubaceae. Other common names includes: English:- bitter ash, bitter damson princess tree, Simarouba, Paradise tree. Spanish:- acajou blanc, daguilla, daguillo gavilan, juan, primero, laguilla, olivio, palo amargo. Creole:- bwa blan, bwa fwenn, doliv fwenn. French:- bois amer, bois blanc, bois frene, bois negresse, quinquina d Europe. Traditional name:- *Simarouba*, Dysentry bark, Mountain Damson, Acituno. (Osagie-Eweka *et al*., 2016). The parts of the plant commonly reportedly used are the leaves, wood and bark. The pharmacological and infact health benefits of many plant tissues are attributed to their inherent bioactive compounds.

Scientific classification

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Clade: Rosids

Order: Sapindales

Family: Simaroubaceae

Genus: *Simarouba*

Species: ***S. glauca DC***

1.7.1 Distribution

S. glauca DC is native to tropical regions of the Americas, specifically Central and South America, including countries like: Guatemala, El Salvador, Honduras, Costa Rica, Colombia,

Venezuela, Peru, Brazil and United States of America (Kumar and Kumar, 2019). It grows in tropical conditions in Central America spreading from Mexico to Panama Southern Florida as well as the Caribbean islands.

1.7.2 Medicinal uses.

i. The leaves and bark have a history of medicinal use in the tropics, particularly in the treatment of malaria, fevers and dysentry (Lakshmi, K.S. *et al*., 2018).

ii. They are also used as a digestive, emmenagogue and to treat parasites both within and on the body.

iii. Studies have shown that the plant is over 90% effective against amoebic dysentry (Lakshmi, K.S. *et al*., 2018).

iv. It contains compounds that help combat oxidative stress, promoting overall health and potentially reducing cancer risk.

v. The quassinoids responsible for the anti-amoebic and antimalarial propeties have also shown to possess active cancer killing properties (Lakshmi, K.S. *et al*., 2018).

vi. It exhibits activity against a range of bacteria, fungi, and parasites, making it useful for infections (Lakshmi, K.S. *et al*., 2018).

vii. The bark is used as a bitter tonic.

viii. Some studies suggests that it may inhibit the growth of cancer cells, especially in breast, colon and liver cancers (Lakshmi, K.S. *et al*., 2018).

ix. They protect the stomach lining, reducing the risks of ulcer and aiding digestion.

x. A decoction is taken internally in the treatment of diarrhoea, dysentry, malaria, fever, haemorrhages, intestinal parasites and colitis (Lakshmi, K.S. *et al*., 2018).

1.7.3 Agroforestry Uses.

i. The deep root system helps in improving soil fertility by bringing up nutrients from lower soil layers (Singh and Garg, 2021).

ii. The oil cake being rich in nitrogen (8%), phosphorus (1,1%) and potash (1.2%) is good organic manure.

iii. It supports biodiversity by providing food and shelter for various birds and insects, contributing to a balanced eco system (Singh and Garg, 2021).

iv. The pulp and leaf litter can be economically used in the manufacture of vermicompost (about 8tons/ha/year).

v. It absorbs significant amount of CO_2 helping to mitigate climate change effects (Singh and Garg, 2021).

vi. It is found as an associated species of the sub tropical moist forest, sharing a position with other common trees of the home and humid perenial gardens, such as Mango (*Mangifera indica*), Plantain (*Musa paradisiaca*) and Royal palm (*Roystonea borinquena*) (Singh and Garg, 2021).

1.7.4 Other Uses.

i. The wood of Simarouba glauca is lightweight, durable, and resistant to decay, making it suitable for furniture, cabinetry, and construction (Ramasamy, S.P. *et al*., 2022).

ii. Oil and extracts from the tree are incorporated into skincare and haircare products for their moisturising and healing properties.

iii. Some studies suggest that extracts from the tree may repel insects, making it a candidate for natural pest control (Ramasamy, S.P. *et al*., 2022).

iv. The wood is lightweight and durable, suitable for furniture, construction, and plywood manufacturing (Ramasamy, S.P. *et al*., 2022).

v. The wood is used for interior construction, boxes and crates, furniture components, veneer and plywood, pattern making, millwork, particleboard and fibreboard.

vi. The lustre is rather high; the texture medium and uniform; the grain usually straight; without odour but with a bitter quinine-like taste (Ramasamy, S.P. *et al*., 2022).

vii. The wood of Simarouba glauca is lightweight, durable, and resistant to decay, making it suitable for furniture, cabinetry, and construction (Ramasamy, S.P. *et al*., 2022).

1.8 AIM OF THE STUDY

Evaluate the efficacy of the function of Losartan/Metformin or Losartan/Glibenclamide on L-NAME of streptozotocin-induced Hypertension/Diabetes in male wister rats.

1.9 OBJECTIVE OF THE STUDY

i. To establish a reliable experimental model of hypertension and type 2 diabetes comorbidity using L-NAME and Streptozotocin-induced pathologies in laboratory animals.

ii. Evaluate the Plasma and Heart High-density Lipoprotein Cholesterol of Hypertensive and Diabetic male wister rat treated with Losartan/Metformin.

iii. Evaluate the Plasma and Heart Low-density Lipoprotein Cholesterol of Hypertensive and Diabetic male wister rat treated with Losartan/Metformin..

iv. Evaluate the Plasma and Heart Total Cholesterol of Hypertensive and Diabetic male wister rat treated with Losartan/Metformin.

v. Evaluate the Plasma and Heart Triglycerides of Hypertensive and Diabetic male wister rat treated with Losartan/Metformin.

vi. To determine the effects of Losartan/Metformin on plasma lipid profile.

CHAPTER TWO

2 MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 COLLECTION OF *SIMAROUBA GLAUCA* LEAVES

Fresh *Simarouba glauca* leaves were collected at Cercobela Farms in Ubiaja, Edo State's Esan South East Local Government Area. Nigeria. A new plant specimen was verified with voucher N0. UBHS382 and placed at the Department of Plant Biology and Biotechnology Herbarium, University of Benin, Benin City, Nigeria. At the University of Benin's Department of Biochemistry, the leaves were air-dried for twenty-eight (28) days after being cleaned with distilled water. The leaves were ground into a fine powder at the Department of Pharmacology, Faculty of Pharmacy, University of Benin, using the extraction technique previously outlined by Osagie Eweka *et al*., (2016).

2.1.2 PREPARATION OF HYDRO-METHANOL FRACTION OF *SIMAROUBA GLAUCA.*

For 48 hours, the powdered *Simarouba glauca* leaf must be macerated in hydromethanol. A mixture of 2.5L hydro-methanol, 20% distilled water, and 80% methanol was used to soak 500g of leaf powder. agitated in a vortex every two hours for the first twenty-four hours. The portion was sieved using a Muslim cloth and then re-submerged for the final 24 hours. In order to obtain a finely powdered hydro-methanol fraction of *Simarouba glauca* (HMFSG), filtrate portions of the hydro-methanol extract of *Simarouba glauca* were pooled and freeze-dried at the Department of Basic Medical Studies, University of Benin, Trigas lab, Benin city, after 48 hours. Following extraction and freeze-drying, 57g was obtained with a percentage yield of 11.4% w/w extraction.

2.1.3 PREPARATION OF ACETONE FRACTION OF SIMARUOBA GLAUCA (AFSM)

For 48 hours, the powdered *Simarouba glauca* leaf must be macerated in acetone. Two litres of acetone were mixed with 500 grammes of leaf powder. agitated in a vortex every two hours for the first twenty-four hours. The portion was seived using a Muslim cloth and then re-submerged for the final 24 hours. To obtain a fine powdered acetone fraction of *Simarouba glauca* (AFSG), filtrate portions of the acetone fraction of *Simarouba glauca* were pooled and freeze-dried at the Department of Basic Medical Studies, University of Benin, Trigas lab, Benin city, after 48 hours. 39g was obtained with a percentage yield of 7.8% w/w extraction following extraction and freeze-drying.

2.2 CHEMICALS/REAGENTS

- L-NAME 250 mg
- Streptozotocin 50mg
- Troponin-I
- NADPH oxidase
- Glucose-6-phosphate dehydrogenase enzyme
- Cholesterol
- HDL
- Triglyceride
- Potassium
- Sodium
- Creatinine
- Urea
- Distilled water

- Methanol
- Acetone solvents
- Picric acid
- Urethane
- Glibenclamide
- Lorsatan
- Metformin

2.3 EQUIPMENTS/APPARATUS

- Sample bottles (EDTA & Lithium heparin)
- Universal bottle
- Sensitive balance
- Wister rat (male)
- Dissecting set
- Water bath
- Spectrophotometer
- Syringe
- Hand gloves
- Face mask
- Foil paper
- Test tubes
- Man pipette
- Glucometer

2.3 Animals

Fifty-two male albino rats (Wistar strain) weighing 50-80 g were procured from the Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

Rats were housed in clean cages with a 12-hour light/dark cycle.

Bedding was replaced daily, and animals were acclimatized for two weeks.

They were fed guinea pellets (Premier Feed Mills Co. Ltd, Ibadan, Oyo State) and provided water ad libitum.

The experimental protocol adhered to guidelines outlined in the Care and Use of Laboratory Animals.

2.4 Methods

2.4.1 Animal Grouping and Administration of Extracts

The 52 rats were divided into six groups (n=6 per group):

Group 1: Normotensive/Non-diabetic (positive control)

Group 2: Hypertensive/Diabetic (negative control)

Group 3: Hypertensive/Diabetic+ treated with losartan/metformin

Group 4: Hypertensive/Diabetic+ treated with losartan/glibenclamide

2.4.2 Induction of Hypertension and Diabetes

1. Hypertension Induction:

Hypertension was induced by administering L-NAME (Nω-Nitro-L-arginine methyl ester) at 40 mg/kg body weight in drinking water for four weeks. L-NAME inhibits nitric oxide synthesis, resulting in increased vascular resistance and hypertension.

2. Diabetes Induction:

Diabetes was induced via a single intraperitoneal injection of streptozotocin (STZ) at 50 mg/kg body weight. STZ selectively destroys pancreatic beta cells, resulting in hyperglycemia.

Successful induction of diabetes was confirmed with fasting blood glucose levels exceeding 200 mg/dL.

Hypertension was confirmed by measuring systolic blood pressure using a non-invasive tail-cuff method.

2.4.3 Animal Sacrifice and Sample Collection

At the end of the 28-day treatment period:

1. Rats were sacrificed via cervical dislocation.

2. Blood samples were collected via cardiac puncture into EDTA-coated tubes.

3. Plasma was separated by centrifugation at 3,000 rpm for 10 minutes and stored at −20°C until GGT analysis.

4. Liver tissues were excised, rinsed in phosphate-buffered saline (PBS), and homogenized. The homogenates were centrifuged, and the supernatants were collected for liver GGT activity analysis.

2.5 Biochemical Assays

2.5.1 Total Cholesterol Assay

This assay is based on an enzymatic colorimetric method using the CHOD-PAP (Cholesterol Oxidase-Phenol Aminophenazone) reaction (Lian, K. *et al,* 2014). The reaction follows these steps:

1. Hydrolysis: Cholesterol esters are hydrolyzed into free cholesterol and fatty acids by Cholesterol Esterase (CE).

2. Oxidation: Free cholesterol is oxidized by Cholesterol Oxidase (CO) to produce cholest-4-en-3-one and hydrogen peroxide (H_2O_2).
3. Color Formation: In the presence of Peroxidase (POD), the generated hydrogen peroxide reacts with 4-Aminoantipyrine (AAP) and Phenol, forming a red quinoneimine dye. The intensity of the color is directly proportional to the cholesterol concentration and is measured photometrically at 500 nm (480–520 nm) (Adu, J. *et al,* 2019).
4. Reaction Summary

Cholesterol Ester + H_2O → Cholesterol + Fatty Acid (Cholesterol Esterase)

Cholesterol + O_2 → Cholest-4-en-3-one + H_2O_2 (Cholesterol Oxidase)

H_2O_2 + AAP + Phenol → Quinoneimine Dye + H_2O (Peroxidase)

PROCEDURE

- Pipette the following into test tubes:
    - Blank: 1.0 mL Reagent + 10 µL Distilled Water
    - Standard: 1.0 mL Reagent + 10 µL Cholesterol Standard
    - Sample: 1.0 mL Reagent + 10 µL Sample (Serum/Plasma)
- Mix well and incubate at 37°C for 5-10 minutes.
- Measure absorbance at 500 nm against blank using a spectrophotometer (Beggio, M. *et al*, 2019).

2.5.2 HDL Cholesterol assay

HDL Cholesterol assay is a direct homogeneous enzymatic method designed to measure HDL cholesterol in serum or plasma without the need for a physical separation (precipitation) step. The method relies on the following key points: Selective Masking/Inhibition, Enzymatic Reaction and colorimetric detection (Benitez, S. *et al,* 2025).

Procedure

The following steps represent a typical procedure:

- Allow all reagents, calibrators, and samples to reach room temperature (or the specified assay temperature).
- Prepare any necessary dilutions as per the manufacturer's recommendations.
- Add a measured volume (e.g., 1.0 mL) of HDL reagent and a suitable volume (e.g., 10 µL) of distilled water.
- Add the same volume of reagent plus a measured volume of calibrator.
- Add reagent and an appropriate volume of the serum/plasma sample (often 10 µL).
- Mix the contents thoroughly.
- Incubate the reaction mixture at 37°C (or as specified) for a defined period (typically 5–10 minutes). This incubation allows the masking reagents to inactivate non-HDL lipoproteins and the enzymatic reactions to proceed.
- Measure the absorbance of the reaction mixture at approximately 500 nm using a spectrophotometer (Navar, A. *et al,* 2024).

- Use the blank to zero the instrument.
- Generate a calibration curve using the calibrators.
- Determine the HDL cholesterol concentration in the samples by comparing the measured absorbance with the calibration curve (Giacona, J. *et al*., 2024).

2.5.3 LDL Cholesterol Assay

The assay is based on a direct homogeneous enzymatic method that selectively measures LDL cholesterol by using a combination of reagents which includes: Selective Masking/Inhibition, Enzymatic Reaction Sequence, Hydrolysis, Oxidation and Photometric Measurement (Wolska, A., 2020)

Procedure

The assay can be performed manually or on an automated clinical chemistry analyzer with the following steps.

- Allow all reagents, calibrators, and samples to reach the recommended temperature (usually room temperature or as specified by the kit).
- Prepare any required dilutions according to the manufacturer's instructions.
- Add a measured volume (e.g., 1.0 mL) of LDL reagent plus a small volume (e.g., 10 µL) of distilled water.
  Add the same volume of LDL reagent plus an appropriate volume of the calibrator solution.
  Add the reagent and a defined volume (often around 10 µL) of serum or plasma.

- Mix the contents of each tube thoroughly.
- Incubate the mixtures at 37°C (or the temperature specified in the kit insert) for a set period (typically 5–10 minutes) (Sampson, M. *et al.,* 2020).

During this incubation:

- The masking agents act to suppress the reactivity of non-LDL lipoproteins.
- The enzymatic reactions (hydrolysis, oxidation, and chromogenic reaction) occur sequentially.
- After incubation, measure the absorbance of each tube at approximately 500 nm using a spectrophotometer.
- Use the blank to zero the instrument (Martin, S. *et al*., 2013).

Calculation:

- Generate a calibration curve using the absorbance readings from the calibrators.
- Determine the LDL cholesterol concentration in the samples by comparing their absorbance to the calibration curve.

2.5.4 The triglyceride assay

The triglyceride assay is based on a sequential enzymatic reaction that involves the following key steps: Hydrolysis of Triglycerides, Phosphorylation of Glycerol, Oxidation of Glycerol-3-Phosphate and Color Formation (Huang, C. *et al.,* 2024).

Procedure

The assay can be performed using manual techniques or on an automated clinical chemistry analyzer. Below is a general outline for a manual procedure (Sami, M. *et al.,* 2023).

- Allow all reagents, calibrators, and samples to equilibrate to the recommended temperature (often room temperature or 37°C as specified by the kit).
- Prepare any necessary dilutions following the manufacturer's instructions. Dispense a specified volume of triglyceride reagent (e.g., 1.0 mL) with a small volume (e.g., 10 µL) of distilled water (Sami, M. *et al.,* 2023).
- Dispense the same volume of reagent plus a defined volume of calibrator. Mix the reagent with an appropriate volume (typically around 10 µL) of serum or plasma.
- Mix each tube or cuvette thoroughly (Sami, M. *et al.,* 2023).
- Incubate at the recommended temperature (commonly 37°C) for a specified period (usually 5–10 minutes).
- During this incubation, the sequential enzymatic reactions take place:
    - Lipase hydrolyzes triglycerides to glycerol.
    - Glycerol is converted to glycerol-3-phosphate by glycerol kinase.
    - Glycerol-3-phosphate is oxidized by GPO to yield hydrogen peroxide.
    - Peroxidase catalyzes the reaction between hydrogen peroxide and the chromogen to form the colored dye (Sami, M. *et al.,* 2023).
- Measure the absorbance of the reaction mixture at the appropriate wavelength (typically around 500 nm) using a spectrophotometer (Yazdani, A. *et al.,* 2018).

- Use the blank reading to zero the instrument.
- Generate a calibration curve from the absorbance readings of the calibrators.
- Determine the triglyceride concentration in the samples by comparing their absorbance values with the calibration curve (Yazdani, A. *et al.,* 2018).

2.6 Statistical Analysis

Data were expressed as mean ± SD. Statistical analyses were performed using SPSS (version 21.0).

1. One-way analysis of variance (ANOVA) was used to evaluate differences among groups.
2. Duncan's multiple range test was employed for post hoc comparisons.
3. A p-value ≤ 0.05 was considered statistically significant.

CHAPTER THREE

3 RESULTS

This chapter shows the quantitative results of the comparative effect of the co-administered losartan/metformin or (and) losartan/glibenclamide on the plasma lipid profile of the streptozotocin induced hypertensive/diabetic male wistar rat.

3.1. Plasma Total Cholesterol

The table below shows the plasma total cholesterol in the normal and test animals, with no significant difference between the groups ($p > 0.05$).

Table 3.1: Plasma Total Cholesterol

Group	Conc. (mg/dl)
Norm/ND	572.8 ± 18.1^{a}
Hyp/Dia	597.1 ± 33.0^{a}
L/M	561.2 ± 27.8^{a}
L/G	615 ± 29.8^{b}

Data are in Mean ± SD. Data were all statistically similar ($p > 0.05$).

3.2. Plasma HDL–Cholesterol

The table below shows the plasma HDL–cholesterol in the normal and test animals, with no significant difference between the groups ($p > 0.05$).

Table 3.2: Plasma HDL–Cholesterol

Group	Conc. (mg/dl)
Norm/ND	110.8 ± 2.1 [a]
Hyp/Dia	85.0 ± 19.2[b]
L/M	103.6 ± 15.5[c]
L/D	134.4 ± 7.1[d]

Data are in Mean ± SD. Data were all statistically similar ($p > 0.05$)

3.3. Plasma Triglycerides

The table below shows the plasma triglycerides in the normal and test animals, with no significant difference across the test groups ($p > 0.05$). However, plasma triglyceride level of the Norm/ND rats was significantly lower ($p < 0.05$) than the test groups.

Table 3.3: Plasma Triglycerides

Group	Conc. (mg/dl)
Norm/ND	99.52 ± 5.0 [a]
Hyp/Dia	160.0 ± 27.2 [b]
L/M	145.2 ± 17.7[c]
L/G	180.3 ± 51.0[d]

Data are in Mean ± SD. Data with the same superscripts were statistically similar ($p > 0.05$), while data with different superscripts were significantly different ($p < 0.05$).

3.4 Plasma LDL–Cholesterol

The table below shows the plasma LDL–cholesterol in the normal and test animals, with no significant difference between the groups ($p > 0.05$).

Table 3.4: Plasma LDL–Cholesterol

Group	Conc. (mg/dl)
Norm/ND	442.1 ± 17.9 [a]
Hyp/Dia	480.1 ± 41.7 [b]
L/M	428.6 ± 11.0 [a]
L/D	444.6 ± 28.8 [a]

Data are in Mean ± SD. Data were all statistically similar ($p > 0.05$)

3.5. Cardiac Total Cholesterol

The table below shows the plasma total cholesterol in the normal and test animals, with no significant difference between the groups ($p > 0.05$).

Table 3.5: Cardiac Total Cholesterol

Group	Conc. (mg/dl)
Norm/ND	60.90 ± 11.2 [a]
Hyp/Dia	31.98 ± 4.71 [a]
L/M	40.84 ± 21.5[a]
L/D	33.04 ± 9.63[a]

Values are in Mean ± SD (n = 5). Data were all statistically similar ($p > 0.05$).

3.6. Cardiac HDL–Cholesterol

The table below shows the Cardiac HDL–cholesterol in the normal and test animals, with no significant difference between the groups ($p > 0.05$).

Table 3.6: Cardiac HDL–Cholesterol

Group	Conc. (mg/dl)
Norm/ND	0.55 ± 2.1^{a}
Hyp/Dia	2.90 ± 19.2^{a}
L/M	1.25 ± 15.5^{a}
L/D	2.87 ± 2.71^{a}

Values are in Mean ± SD (n = 5). Data were all statistically similar ($p > 0.05$)

3.7. Cardiac Triglycerides

The table below shows the Cardiac triglycerides in the normal and test animals, with no significant difference across the test groups ($p > 0.05$). However, Cardiac triglyceride level of the Norm/ND rats was significantly lower ($p < 0.05$) than the test groups.

Table 3.7: Cardiac Triglycerides

Group	Conc. (mg/dl)
Norm/ND	39.76 ± 6.99^{a}
Hyp/Dia	27.92 ± 12.55^{b}
L/M	23.30 ± 7.06^{b}
L/D	9.20 ± 2.82^{b}

Values are in Mean ± SD (n = 5). Data with the same superscripts were statistically similar ($p > 0.05$), while data with different superscripts were significantly different ($p < 0.05$).

3.8 Cardiac LDL–Cholesterol

The table below shows the Cardiac LDL–cholesterol in the normal and test animals, with no significant difference between the groups ($p > 0.05$).

Table 3.8: Cardiac LDL–Cholesterol

Group	Conc. (mg/dl)
Norm/ND	51.59 ± 10.98^{a}
Hyp/Dia	23.49 ± 6.26^{b}
L/M	34.93 ± 21.79^{b}
L/D	28.33 ± 11.55^{b}

Values are in Mean ± SD (n = 5). Data with different superscripts are significantly different ($p < 0.05$), while data with the same superscripts are statistically similar ($p > 0.05$)

CHAPTER FOUR

4.1. Discussion

The results of this study provide insight into the comparative effects of co-administration of losartan/metformin (L/M) and losartan/glibenclamide (L/D) on the plasma and cardiac lipid profile of streptozotocin (STZ)-induced hypertensive/diabetic male Wistar rats. The findings are crucial for understanding the rel between these pharmacological agents in modulating lipid metabolism under conditions of comorbid hypertension and diabetes.

Both plasma and cardiac total cholesterol levels were assessed across all groups, with no significant differences observed ($p > 0.05$). The normal/nondiabetic (Norm/ND) group had a mean concentration of 572.8 ± 18.1 and 60.90 ± 11.2 mg/dl, while the hypertensive/diabetic (Hyp/Dia) group presented a slightly elevated levels at 597.1 ± 33.0 and 31.98 ± 4.71 mg/dl respectively. The groups treated with L/M and L/D exhibited concentrations of 561.2 ± 27.8 mg/dl and 615 ± 29.8 mg/dl in the plasma, while cardiac levels were 40.84 ± 21.5 and 33.04 ± 9.63 mg/dl respectively. These results suggest that neither co-administration of L/M nor L/D significantly modulated total cholesterol levels in the test animals. This lack of significant change aligns with previous findings, indicating that losartan alone or in combination with hypoglycemic agents may not substantially influence cholesterol homeostasis in diabetic models (Mishra *et al.*, 2019; Wang *et al.*, 2021). The slight variability in the data could be attributed to inherent metabolic adaptations in the STZ model, as STZ induces hyperglycemia and oxidative stress without directly altering cholesterol synthesis pathways (Ahmed *et al.*, 2020).

In contrast, plasma and cardiac HDL-cholesterol, a critical marker of cardiovascular protection, displayed an interesting trend, albeit not statistically significant ($p > 0.05$). The Norm/ND group had a mean HDL concentration of 110.8 ± 2.1 mg/dl, which decreased in the Hyp/Dia group to 85.0 ± 19.2 mg/dl, indicating dyslipidemia typically associated with diabetes and hypertension (Klein and Sheetz, 2022). The L/M-treated group exhibited a mean cardiac HDL level of 1.25 ± 15.5 mg/dl, suggesting a partial restoration of HDL levels. Surprisingly, the L/D-treated group demonstrated the highest HDL

concentration at 2.87 ± 2.71 mg/dl, a trend that may point to glibenclamide's potential effect on HDL metabolism. While this increase did not achieve statistical significance, suggests that sulfonylureas like glibenclamide may exert favorable effects on HDL levels by modulating hepatic lipase activity (Zhao *et al.*, 2018).

Triglyceride levels, showed a notable pattern, with significant differences observed between groups ($p < 0.05$) in both the plasma and the heart. The Norm/ND had a mean plasma and cardiac triglyceride concentration of 99.52 ± 5.0 mg/dl and 39.76 ± 6.99 mg/dl respectively were lower than Hyp/Dia animals (160.0 ± 27.2 mg/dl and 27.92 ± 12.55 mg/dl). This elevation corroborates the hypertriglyceridemia characteristic of diabetic dyslipidemia, likely driven by insulin resistance and increased hepatic lipogenesis (Zhang *et al.*, 2017). Treatment with L/M and L/D resulted in plasma triglyceride of 145.2 ± 17.7 mg/dl and 180.3 ± 51.0 mg/dl, respectively, while cardiac triglycerides were met at 23.30 ± 7.06 mg/dl and 9.20 ± 2.82 mg/dl. While both treatment groups exhibited reduced levels compared to the Hyp/Dia group, only the L/M-treated group approached statistical similarity with the control group, suggesting that metformin's known effect on reducing hepatic triglyceride synthesis may have contributed to this observation (Tan *et al*., 2021).

The statistical differences observed in triglyceride levels but not in total or HDL cholesterol highlight the differential effects of these pharmacological combinations on lipid subfractions. This divergence shows the need for distinct metabolic pathways affected by antihypertensive and antidiabetic agents. For instance, losartan's ability to modulate lipid metabolism via peroxisome proliferator-activated receptor (PPAR)-γ activation may synergize with metformin's enhancement of AMP-activated protein kinase (AMPK) activity, collectively reducing triglycerides but having a minimal effect on cholesterol levels (Kondo *et al.*, 2016). Conversely, glibenclamide’s impact on triglycerides may be limited by its predominant mechanism of action on insulin secretion without direct lipid-modulating effects (Shao *et al*., 2023).

Plasma and cardiac LDL-cholesterol levels were statistically similar across groups ($p > 0.05$), as indicated in the results. The Norm/ND group had a mean plasma LDL levels of 442.1 ± 17.9 mg/dl, similar to the Hyp/Dia group, which recorded 480.1 ± 41.7 mg/dl. Treatment with losartan/metformin (L/M) resulted in a slightly reduced mean LDL concentration of 428.6 ± 11.0 mg/dl, while losartan/glibenclamide (L/D) produced 444.6 ± 28.8 mg/dl in the plasma. Despite the numerical variations, these differences were not statistically significant, indicating a negligible impact of the treatment regimens on LDL levels.

The absence of significant differences in LDL-cholesterol suggests that the pharmacological effects of losartan and the co-administered antidiabetic agents may not strongly influence LDL metabolism in this experimental model. Losartan, though known to improve vascular function by reducing oxidative stress and inflammation, has limited direct effects on LDL-cholesterol clearance mechanisms (Wei *et al.*, 2018). Metformin, which primarily acts to reduce hepatic gluconeogenesis and improve insulin sensitivity, may not substantially alter LDL receptor expression or function (Viollet *et al.*, 2022).

Glibenclamide, on the other hand, primarily enhances insulin secretion from pancreatic β-cells but lacks significant lipid-modulatory effects, potentially explaining its minimal influence on LDL levels in this context (Cryer, 2016). These findings align with the understanding that LDL-cholesterol is more resistant to modulation by these agents, as their primary mechanisms target glucose and triglyceride metabolism rather than LDL-specific pathways. This highlights the need for adjunct therapies, such as statins, for significant reductions in LDL cholesterol when managing combined metabolic disorders.

4.2. Conclusion

This study demonstrates that while co-administration of losartan with metformin or glibenclamide does not significantly alter plasma and cardiac total or HDL cholesterol levels. It does exerts differential effects on plasma triglycerides in STZ-induced hypertensive/diabetic Wistar rats. These findings contribute to the growing body of evidence on the nuanced effects of antihypertensive and antidiabetic therapies on lipid

metabolism, providing a foundation for further investigations to optimize therapeutic strategies for comorbid conditions.

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APPENDIX

PLASMA TOTAL CHOLESTEROL

NORM/ND	REF BLANK	TEST	Conc (mg/dl)	HYP/DIA	REF BLANK	TEST	Conc (mg/dl)
1	0.123	1.439	581.625	6		1.224	475.2
2	0.264	1.309	517.275	7		1.533	628.155
3		1.531	627.165	8		1.479	601.425
4		1.443	583.605	9		1.626	674.19
5		1.384	554.4	10	P-value	1.489	606.375
Mean			**572.814**		0.538108575		**597.069**
SEM			**18.09518588**				**33.01128559**

L/M	REF BLANK	TEST	Conc (mg/dl)	L/G	REF BLANK	TEST	Conc (mg/dl)
11		1.470	596.97	15		1.580	651.42
12		1.237	481.635	16		1.440	582.12
13		1.404	564.3	17		1.373	548.955
14		1.480	601.92	18		1.633	677.655
	P-value				P-value		
	0.449231492		**561.20625**		0.235119291		**615.0375**
			27.80559052				**29.85394478**

PLASMA HDL CHOLESTEROL @ 500nm

NORM/ND	REF BLANK	TEST	Conc (mg/dl)	HYP/DIA	REF BLANK	TEST	Conc (mg/dl)
1		0.540	106.5	6		0.656	135.5
2	0.114	0.583	117.25	7		0.285	42.75

3		0.570	114	8		0.456	85.5
4		0.554	110	9		0.592	119.5
5		0.540	106.5	10	P-value	0.281	41.75
Mean			**110.85**		0.218256407		**85**
SEM			**2.111116298**				**19.19691522**

L/M	REF BLANK	TEST	Conc (mg/dl)	L/G	REF BLANK	TEST	Conc (mg/dl)
11		0.584	117.5	15		0.615	125.25
12		0.370	64	16		0.601	121.75
13		0.500	96.5	17		0.663	137.25
14		0.659	136.25	18		0.727	153.25
	P-value				P-value		
	0.493539093		**103.5625**		0.060297817		**134.375**
			15.48600679				**7.113294478**

PLASMA TRIGLYCERIDE @ 500nm

NORM/ND	REF BLANK	TEST	Conc (mg/dl)	HYP/DIA	REF BLANK	TEST	Conc (mg/dl)
1	0.297	0.268	107.2	6		0.268	107.2
2		0.206	82.4	7.00		0.398	159.2
3		0.271	108.4	8		0.270	108
4		0.234	93.6	9		0.640	256
5		0.265	106	10	P-value	0.424	169.6

Mean			**99.52**		0.030145843		**160**
SEM			**5.030836036**				**27.155777**

L/M	REF BLANK	TEST	Conc (mg/dl)	L/G	REF BLANK	TEST	Conc (mg/dl)
11		0.473	189.2	15		0.441	176.4
12		0.271	108.4	16		0.809	323.6
13		0.317	126.8	17		0.221	88.4
14		0.391	156.4	18		0.332	132.8
	P-value				P-value		
	0.681147065		**145.2**		0.539845515		**180.3**
			17.68766048				**51.03263662**

NORM/ ND	Conc (mg/dl)	HYP/DI A	Conc (mg/dl)	L/M	Conc (mg/dl)	L/G	Conc (mg/dl)
1	453.685	6	318.26	11	441.63	15	490.89
2	383.545	7	553.565	12	395.955	16	395.65
3	491.485	8	494.325	13	442.44	17	394.025
4	454.885	9	503.49	14	434.39	18	497.845
5	426.7	10	530.705				
Mean	**442.06**		**480.069**		**428.6037 5**		**444.6025**
SEM	**17.86047 221**		**41.69962 835**		**11.03232 587**		**28.76880 142**
		P-value		P-value		P-value	
		0.213601 434		0.161219 742		0.311089 991	

Plasma LDL-Cholesterol

CARDIAC TOTAL CHOLESTEROL

NORM/ND	REF BLANK	TEST	Conc (mg/dl)	HYP/DIA	REF BLANK	TEST	Conc (mg/dl)
1	0.088	0.291	100.485	6		0.158	34.65
2	0.073	0.185	48.015	7		0.142	26.73
3		0.195	52.965	8		0.121	16.335
4		0.219	64.845	9		0.172	41.58
5		0.157	34.155	10	P-value	0.17	40.59
Mean			**60.093**		0.049883802		**31.977**
SEM			**11.21318354**				**4.712674111**

L/M	REF BLANK	TEST	Conc (mg/dl)	L/G	REF BLANK	TEST	Conc (mg/dl)
11		0.11	10.89	15		0.113	19.8
12		0.153	32.175	16		0.192	51.48
13		0.121	16.335	17		0.115	13.365
14		0.298	103.95	18		0.184	47.52
	P-value				P-value		
	0.665553592		**40.8375**		0.752082022		**33.04125**
			21.51637327				**9.626816793**

CARDIAC HDL–CHOLESTEROL

NORM/ND	REF BLANK	TEST	Conc (mg/dl)	HYP/DIA	REF BLANK	TEST	Conc (mg/dl)
1		0.119	1.5	6		0.116	0.75
2	0.113	0.113	0	7		0.114	0.25
3		0.114	0.25	8		0.189	14.25
4		0.112	-0.25	9		0.113	0
5		0.118	1.25	10	P-value	0.11	-0.75
Mean			**0.55**		0.436450675		**2.9**
SEM			**0.347598471**				**2.842807228**

L/M	REF BLANK	TEST	Conc (mg/dl)	L/G	REF BLANK	TEST	Conc (mg/dl)
11		0.118	1.25	15		0.157	11
12		0.112	-0.25	16		0.114	0.25
13		0.128	3.75	17		0.113	0
14		0.114	0.25	18		0.114	0.25
	P-value				P-value		
	0.634657653		**1.25**		0.29471399		**2.875**
			0.889756521				**2.708974283**

CARDIAC TRIGLYCERIDE

NORM/ND	REF BLANK	TEST	Conc (mg/dl)	HYP/DIA	REF BLANK	TEST	Conc (mg/dl)
1	0.114	0.218	41.6	6		0.281	66.8
2		0.257	57.2	7.00		0.13	6.4
3		0.244	52	8		0.121	2.8
4		0.183	27.6	9		0.153	15.6
5		0.165	20.4	10	P-value	0.234	48
Mean			**39.76**		0.217214682		**27.92**
SEM			**6.987826514**				**12.54926516**

L/M	REF BLANK	TEST	Conc (mg/dl)	L/G	REF BLANK	TEST	Conc (mg/dl)
11		0.215	40.4	15		0.145	12.4
12		0.152	15.2	16		0.135	8.4
13		0.136	8.8	17		0.15	14.4
14		0.186	28.8	18		0.118	1.6
	P-value				P-value		
	0.774948019		**23.3**		0.113189317		**9.2**
			7.062341444				**2.823709145**

Cardiac LDL-Cholesterol

NORM/ND	Conc (mg/dl)	HYP/DIA	Conc (mg/dl)	L/M	Conc (mg/dl)	L/G	Conc (mg/dl)
1	90.665	6	20.54	11	1.56	15	6.32
2	36.575	7	25.2	12	29.385	16	49.55
3	42.315	8	1.525	13	10.825	17	10.485
4	59.575	9	38.46	14	97.94	18	46.95
5	28.825	10	31.74				
Mean	**51.591**		**23.493**		**34.9275**		**28.32625**
SEM	**10.98145532**		**6.258966702**		**21.78610992**		**11.54656106**
		P-value		P-value		P-value	
		0.028633059		0.296419007		0.398938157	