INVESTIGATION OF THE EFFECT OF ETHANOL EXTRACT OF *VERNONIA AMYGDALINA* LEAVES ON CARDIOVASCULAR DISEASE RISK FACTORS IN RATS EXPOSED TO DMH

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A FINAL YEAR PROJECT SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY.

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR A BACHELOR OF SCIENCE (B.Sc.) IN BIOCHEMISTRY.

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CERTIFICATION

We the undersigned, hereby certify that this research work was carried out by AKASHILI EMMAUNEL with the matriculation number LSC1806247 in the department of Biochemistry, University of Benin and that this work is considered adequate for consideration in partial fulfillment of the requirement for the award of Bachelor of Science in Biochemistry.

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DEDICATION

This project work is dedicated to God Almighty for His Love and mercies. All the glory belongs to Him alone for the success of this work.

ACKNOWLEDGEMENT

I am grateful to the Almighty God for His grace and mercies throughout this research work. I also want to acknowledge my project supervisor, and my lecturer Dr OSAHON D. ABU for his guidance to making this project a success.

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ABSTRACT

Vernonia amygdalina (Bitter leaf) is a medicinal plant used in traditional medicine for the treatment of various diseases. It possesses different biological activity including antioxidant and antimicrobial activities, antibacterial and analgesic activity. The aim of this study was to investigate the effect of *Vernonia amygdalina* on cardiovascular disease risk factors in rats exposed to DMH. Adult Wister rats (n= 30) were collected and randomly assigned to groups (8 rats per groups): normal control, DMH only, Silymarin control, extract only, post treatment 1, post treatment 2, Pre- treatment 1, and Pre- treatment 2. Group 1, was supplied with food and water regularly but no Vernonia amygdalina extract, silymarin antibiotic or 1,2 dimethylhydrazine was administered. Group 2, 1,2 dimethylhydrazine was administered at 3millilitres per kilograms of body, alongside with food and water to induce the heart damage. Loss of appetite, fatigue and dizziness was noted after few days of administration. Group 3, the silymarin an antibiotic at 100ml\kg by weight was administered, DMH was then administered every day for 12 days. Concentrations of lipids: total cholesterol, HDL cholesterol, TAG, High and low-Density lipoprotein cholesterol and very low-Density lipoprotein cholesterol as well as Atherogenic index, Atherogenic coefficient, cardiac risk ratio and cardiac Nitric Oxide was measured in plasma. The result show that the exposure of normal Wistar rats to a single intraperitoneal dose of DMH significantly elevated their total protein (TP), lipid profile [total cholesterol (TC), triacylglycerol (TG), very-low density lipoprotein cholesterol (VLDL-C), low-density lipoprotein cholesterol (LDL-C)], and cardiovascular disease risk factors [atherogenic index of plasma (AIP), atherogenic coefficient (AC), cardiac risk ratio (CRR)], but these parameters were markedly reduced after treatment with ethanol extract of the medicinal plant (*p*

< 0.05). In addition, the extract significantly elevated high-density lipoprotein cholesterol (HDL-C) level which was reduced by DMH ($p < 0.05$

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Plants are rich in bioactive compounds, fibers, and essential nutrients, providing a variety of health benefits. These benefits extend to, physical, mental, and emotional well-being. Plants contains various bioactive compounds, including polyphenols, carotenoids, and phytosterol, which possess antioxidant, anti-inflammatory, and immunomodulatory properties. These compounds can help mitigate the risk of diseases such as cancer, cardiovascular diseases, diabetes, and neurodegenerative disorders. They do so by improving intestinal barrier function and reducing inflammation in the body (Samtiya*, et al*, 2021; Leri *et al*, 2020; El-Ramady *et al*, 2022). Plants have long been used for their medicinal benefits (Simone Rochfort *et al*, 2008). Addressing chronic diseases is a critical challenge for the coming decades. Plant-based foods contain a wide range of metabolites, many of which are known to promote human health. Analyzing the composition of these metabolites through plant biochemistry can provide valuable insights that contribute to improving human health (Cathie Martin *et al,* 2011). Plants are becoming a common source for producing high-quality biological molecules used in pharmaceuticals and industrial biomaterials (Gargana and Zahmanova, *et al*, 2023). People in both developing and developed countries rely on medicinal plants for their healthcare needs. These plants are used to maintain health and treat illnesses. (Carsten Smith-Hall *et al*, 2012).

1.1 Aim and Objectives of the Study

1. To evaluate the therapeutic effect of Vernonia amygdalin leaf extraction in cardiovascular disease risk in rats exposed to DMH.

2. To extract the leaf of *Vernonia amygdalina* with absolute ethanol.

3. To fractionate the crude ethanol of *Vernonia amygdalin* using solvents of increasing polarity.

4. To induce DMH in normal Wister rats.

5. To induce DMH in normal Wister rats using STZ.

6. To treat the cardiac disease rats with ethanol extract of the plant fractions.

1.2 Literature Review

1.2.1 Botanical Description of *Vernonia amygdalin*

Vernonia amygdalina, commonly known as Bitter Leaf, is a plant that belongs to the Asteraceae family. There is some debate about its classification, with some suggesting itshould be placed under the genus Gymnanthemum Cass. instead of Vernonia Shrub (Mao *et al*, 2023). Vernonia amygdalina is a tropical shrub that can grow up to 10 meters tall. (Jayaweera *et al* 2021; Pol *et al,* 2024). The plant is characterized by its bitter-tasting leaves, which are a distinctive feature (Jayaweera et al, 2021; Yeap *et al*, 2010). The plant thrives in tropical regions with an average rainfall of 750-2000 millimeters. It can be found near rivers, lakes, forests, and grasslands up to an elevation of 2800 meters (Erasto *et al*, 2007). This plant is primarily found in Tropical Africa. It has a wide range of medicinal uses, including the treatment of diabetes, malaria, gastrointestinal issues, stomachaches, skin infections, and pneumonia. Additionally, the plant exhibits anti-cancer properties (Bhavana Joshi, G. Panwar, and S.K. Singh *et al*,2019). The plant

known for its bitter-tasting leaves, which are commonly used as a vegetable in many African countries. The tree has delicate, slender branches and is often considered a small tree due to its modest height (Pol *et al*, 2024). “It is nutritionally rich, containing proteins, fats, fibers, amino acids, minerals, vitamins, and carbohydrates (Mao *et al*, 2023; Degu *et al*, 2024).

Figure 1.1 Pictorial representation of *Vernonia amygdalina*

Table 1.1 Taxonomical classification of *Vernonia amygdalina*

Kingdom	Plantae
Class	Angiospermae
Order	Asterales
Family	Asteraceae
Genus	Vernonia
Species	Amygdalina

1.2.2 Ecology

Veronica amygdalina is a plant species that is commonly found in the following natural habitats: - Near rivers and lakes - In forest margins - In woodlands - In grasslands – At elevations up to 2,800 meters - In areas with an average annual rainfall of 750-2,000 mm.

1.2.3 Distribution

Vernonia amygdalina, a plant native to Africa, is extensively used in traditional medicine across the continent. It is particularly prevalent in Nigeria, where it is commonly used to treat diseases such as malaria and diabetes. (Nworji *et al*, 2023). The plant has spread to Asia, including Indonesia, where it is known locally as the African plant. It is used for its medicinal properties, particularly in the treatment of diabetes and hypertension. (Erwin *et al*, 2022). Vernonia amygdalina is primarily found in Africa and parts of Asia. However, the broader genus Vernonia, which includes different species, has been studied in other regions such as New Zealand. These studies, however, have focused on species other than *Vernonia amygdalina* (Aet4612 *et al*, 2022).

1.2.4 Non medicinal used Vernonia amygdalina

Vernonia amygdalina, commonly known as bitter leaf, has diverse applications beyond its traditional medicinal uses. This plant's extracts and essential oils are increasingly being utilized in various industries, including brewing, agriculture, and cosmetics, showcasing its versatility. The plant's leaves are nutrient-rich, improving food security and the nutritional value of agricultural products. (Degu *et al*, 2024). *Vernonia amygdalina*, a plant commonly known as bitter leaf, has shown promising potential in the brewing industry. Methanol extracts derived from this plant have demonstrated the ability to inhibit the growth of certain microorganisms, while simultaneously promoting the growth of yeast. This unique property makes *Vernonia*

amygdalina a viable alternative to hops, a traditional ingredient used in brewing. Fermentation of sugars using *Vernonia amygdalina* (Bitter leaf) extracts can yield alcohol concentrations ranging from 4.06% to 5.15%, indicating the effectiveness of this method for alcohol production. (Babalola & Okoh *et al*, 1996). The antimicrobial properties of Vernonia amygdalina extracts can be utilized in organic farming as natural pesticides, aligning with the growing consumer preference for organic products. The antimicrobial and antioxidant properties of V. amygdalina make it a suitable ingredient for cosmetic and hygiene products. These properties can address various beauty concerns, such as: 1. Antimicrobial effects: The antimicrobial properties of *V. amygdalina* can help protect the skin from harmful microorganisms, making it beneficial for products targeting acne, blemishes, or other skin infections. 2. Antioxidant activity: The antioxidant properties of V. amygdalina can help neutralize free radicals, which can contribute to skin aging and damage. This makes it useful for anti-aging and skin rejuvenation products. 3. Versatility: The multifunctional nature of V. amygdalina allows it to be incorporated into a wide range of cosmetic and hygiene products, such as cleansers, moisturizers, serums, and personal care items, to address various beauty concerns. By leveraging the antimicrobial and antioxidant properties of V. amygdalina, cosmetic and hygiene product manufacturers can develop formulations that cater to the diverse needs of consumers, promoting healthy and radiant skin (Bolouri *et al*., 2022).

1.2.5 Ethno -medicinal uses of Vernonia amygdalin

Vernonia amygdalina is a widely used traditional medicinal plant, particularly in Africa. The plant's leaves can be prepared and consumed in the following ways: 1. Raw consumption: The fresh leaves can be eaten without any further processing. 2. Cooking: The leaves can be cooked, either on their own or as an ingredient in a dish. 3. Juicing: The leaves can be processed into

juices. The specific methods of preparation and administration may vary across different cultural and regional practices. Fresh leaves are squeezed to extract juice, which is consumed for its health benefits. The juice is particularly beneficial for gastrointestinal issues and fever (Ugbogu *et al*,2021). Leaves are commonly used as ingredients in soups and stews. This not only enhances the flavor of the dish but also helps preserve some of the leaves' medicinal properties. The juice has antimicrobial properties, so it is sometimes applied directly to wounds or skin infections. (Ogwu & Ikhajiagbe, *et al* 2023). In certain cultures, bitter leaf is combined with other herbs to amplify its medicinal benefits, especially for treating complex health issues. (Egharevba *et al*, 2014).

1.2.6. Biological activities of Vernonia amygdalin

Vernonia amygdalina, commonly known as bitter leaf, has a wide range of biological activities that suggest it has numerous therapeutic applications. Its rich phytochemical profile, which includes terpenoids, flavonoids, and alkaloids, contributes to its effectiveness in treating various health conditions. The following sections outline its potential therapeutic uses based on recent research findings.

1.2.7. Anti-ulcer activity

The aqueous extract of V. amygdalina showed a significant ulcer inhibition rate of 76% at a dosage of 500 mg/kg, which is comparable to the 79% inhibition rate observed with the use of cimetidine. This suggests that the extract has the potential to be an effective therapeutic agent for the treatment of hydrochloric acid/ethanol-induced gastric ulcers, as it was able to effectively reduce the ulcer indices in this model (Azubuike *et al*., 2022).

1.2.8. Antioxidant and Antimicrobial activity

Vernonia amygdalina is known for its potent antioxidant properties, which can be attributed to its rich content of flavonoids and polyphenols. These compounds effectively neutralize free radicals, thereby mitigating oxidative stress. Researchers have employed various extraction methods, such as using methanol and aqueous solvents, to obtain extracts from Vernonia amygdalina. These extracts have demonstrated significant radical scavenging abilities, as evidenced by their low IC50 values in DPPH and ABTS assays. This indicates a high antioxidant capacity. Among the different fractions, the dichloromethane fraction has been found to have the highest concentration of flavonoids and the greatest antioxidant activity. (ROSALINA *et al*., 2024)

Flavonoids are able to donate electrons to free radicals, which helps to stabilize the free radicals and prevent cellular damage. These compounds increase the reducing ability of the system and contribute to its overall antioxidant capacity. (Wang *et al*., 2020). The effectiveness of plant extracts is commonly evaluated using DPPH and ABTS assays. These assays measure the antioxidant capacity of the extracts. Methanol extracts tend to exhibit superior antioxidant activity compared to other solvents, as they typically contain higher concentrations of phenolic compounds, which are the primary contributors to the antioxidant properties of the extracts. (Ahmed and Endalew *et al*, 2023). Vernonia amygdalina, a plant commonly known as bitter leaf, has been found to possess antioxidant properties that are comparable to those of other natural sources, such as green tea and berries. This Vernonia amygdalina, a natural food source, is rich in

flavonoids and polyphenols, which are known to have antioxidant properties. Unlike some antioxidants that are primarily composed of a single type of compound*, V. amygdalina* contains a diverse array of bioactive compounds, which enhances its overall effectiveness as an antioxidant (DJATAOU *et al,* 2024).

1.2.9. Antibacterial activity

Vernonia amygdalina extract contains compounds rich in various phytochemicals. Phytochemical screening of the extracts revealed variations in their composition, with the presence or absence of certain components. The presence of glycosides, alkaloids, and flavonoid is believed to contribute to the antibiotic properties of V. amygdalina leaves and confirm their antimicrobial efficacy against selected pathogens. The extract concentrations ranging from 50 mg/mL to 1.56 mg/mL were able to inhibit the growth of epidermidis. The Minimum Inhibitory Concentration (MIC) is the smallest concentration that can inhibit bacterial growth (Mahatir, D.L. Effendy and C. Henni *et al*,2023).

1.3. Molluscicidal activity

Vernonia amygdalina, a plant species, possesses molluscicide (snail-killing) properties primarily due to its chemical composition. The key phytochemicals responsible for this activity are saponins, glycosides, and phenolic compounds. These compounds have shown potent effectiveness against freshwater snails, which act as intermediate hosts for the parasitic infection schistosomiasis. Glycosides are plant-derived compounds that contribute to the overall bioactivity of the plant, including enhancing its molluscicide (snail-killing) effects. (Otwani *et al*, 2017).

1.3.1 Analgesic activity

The analgesic (pain-relieving) properties of Vernonia amygdalina can be attributed to its diverse phytochemical constituents, which include various bioactive compounds. These compounds have demonstrated significant pharmacological effects, particularly in pain relief and anti-

inflammatory activities. The following sections will provide a detailed overview of the key phytochemicals responsible for the analgesic properties of this plant. Flavonoids: Luteolin and apigenin are compounds that have been shown to have anti-inflammatory and analgesic (pain-relieving) properties. These effects can help alleviate pain. Tannins: Tannins, when present in significant amounts, have been associated with analgesic (pain-relieving) properties. This is because tannins can modulate or influence pain pathways in the body. Alkaloids: The compounds, such as cryptolepine and isocryptolepine, are known for analgesic (pain-relieving) properties, which affect the central nervous system. (Ugbogu *et al*, 2021, Audu *et al*, 2012).

1.3.2 Anti-plasmodial activity

The anti-malarial properties of Vernonia amygdalina can be attributed to several mechanisms, primarily involving its bioactive compounds and their effects on the Plasmodium parasite. Studies have shown that the ethanolic extracts of *V. amygdalina* exhibit significant curative effects against Plasmodium berghei, with the efficacy increasing in a dose-dependent manner. (Joseph *et al*, 2020) (Omoregie and Pal *et al*, 2016).

2. Dimethylhydrazine (DMH) Overview

Dimethylhydrazine (DMH) is a compound with important implications in environmental and health contexts. It is known for its ability to cause colorectal cancer and its use as a component in rocket fuel, which raises concerns about its toxicity and environmental impact. The subsequent sections will provide detailed information on the health effects of DMH, environmental concerns related to its use, and analytical methods for its detection. Experimental studies have shown that DMH (a chemical compound) can induce morphological changes and inflammation in the colonic mucosa, ultimately leading to the development of colorectal cancer. (Paramita *et al*,

2024). Unsymmetrical dimethylhydrazine (UDMH), a type of dimethylhydrazine (DMH), has been shown to have harmful effects on the heart in rats. Specifically, subchronic (long-term) exposure to UDMH has led to significant changes in heart function in these animals. (Ukolov *et al*, 2022). UDMH is highly soluble in water and poses health risks due to chronic exposure. Prolonged exposure can lead to physiological and biochemical changes in Organisms (Maslennikov *et al*, 2022). The compound's presence in the environment necessitates effective removal strategies. Novel porous carbons have effectively removed UDMH (unsymmetrical dimethylhydrazine) from wastewater, underscoring the need for efficient environmental remediation strategies. (Myhill *et al*, 2022). 1,1-Dimethylhydrazine (UDMH): Rocket propellant due to high energy density and performance. (Keshavarz *et al*, 2011). It Involved in the production of diverse chemical products and materials (Lopyrev *et al*,1998). Derivatives of this compound are useful in purification processes, such as removing formaldehyde dimethyl hydrazone from solutions. (Nauflett *et al*, 1978). N, N-Dimethylhydrazine: This compound serves as a versatile reagent in organic synthesis. It is commonly used to prepare N, N-dimethyl hydrazones and functionalized Wittig reagents." It directly states the key applications of the compound, without unnecessary details or jargon. (Winter *et al*, 2007). As a nucleophile, it can react with various electrophiles, making it a versatile reagent forchemical transformations.

1.3.4. Toxicity overview

Toxicity refers to the ability of a substance to disrupt metabolic processes, potentially leading to physiological disorders or even death (Ngounou *et al*, 2020). Toxicology is the study of the harmful effects of chemical, physical, or biological agents on individual organisms and ecosystems (Pope *et al*, 2020). Factors Influencing Toxicity Physical and Chemical Properties Particle size and zeta potential influence toxicity. Smaller particles and those with a positive zeta

potential exhibit higher toxicity. This is due to increased cellular uptake and interaction with the negatively charged cell membranes (Płuciennik *et al*, 2024). The chemical structure of xenobiotics, including their size, shape, and surface area, significantly impacts their toxicity. These structural properties influence the absorption and metabolism of the xenobiotics (Gupta *et al*, 2022).

Biological Factors

Physiological Variability: Individual responses to drugs can vary due to factors such as age, gender, and hormonal conditions, which can affect drug metabolism and toxicity (Gupta *et al*, 2022). Cell Type Sensitivity: Different cell types respond variably to toxic substances, with normal cells often being more sensitive than cancerous ones (Płuciennik *et al*, 2024).

Environmental Influences

Environmental Conditions: Environmental factors like salinity, sunlight, and temperature can alter the behavior and toxicity of chemicals, including pesticides. ("Influence of Salinity, Sunlight, and Sediment on the Toxicity of Pesticides in Three Non-Target Organisms", 2022). Exposure Duration: Prolonged exposure to toxic substances can lead to adverse effects, as observed in biological wastewater treatment processes. (Xu & Zheng *et al*, 2021).

1.3.5. Heart Overview

The heart is a vital organ that serves as a pump, circulating blood throughout the body. Beyond its physical function, the heart also holds significant cultural and emotional importance. Its role extends beyond physical health, influencing various philosophical and spiritual beliefs. This multifaceted nature of the heart can be explored through three key aspects: 1. Anatomical functions: The heart's role as a circulatory pump. 2. Heart transplantation: The medical and

symbolic significance of this procedure. 3. Cultural symbolism: The heart's representation in diverse belief systems and traditions. By examining these three elements, we can gain a comprehensive understanding of the heart's importance, both physiologically and in the human experience.

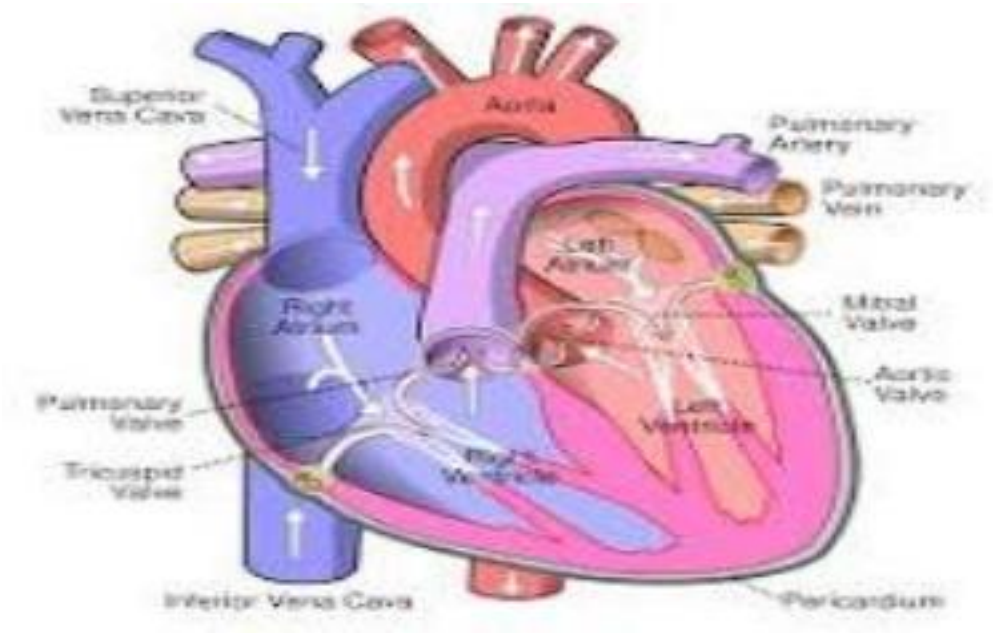

Fig 1.2 Pictorial representation of the heart.

1.3.6. Anatomy

The heart is a complex and vital organ responsible for circulating blood throughout the body. It is composed of four main chambers: the right and left atria, and the right and left ventricles. These chambers work together to ensure efficient blood flow. Understanding the heart's anatomy is crucial for both clinical practice and surgical interventions, as it guides the management of various cardiovascular conditions.

1.3.7. Heart Wall's Layers

The heart wall is composed of three distinct layers: 1. Endocardium: This innermost layer lines the heart's chambers, providing a smooth surface for blood flow. It contains endothelial cells that regulate vascular functions. 2. Myocardium: This is the thickest layer, consisting of cardiac muscle cells (cardiomyocytes) that contract to pump blood. The complex arrangement of these cells allows for efficient force generation and transmission.3. Epicardium (or visceral pericardium): This protective outer layer contains blood vessels that supply the myocardium.

1.3.8. Structure and function

The heart's function is driven by a coordinated sequence of electrical impulses that trigger the contractions of the heart muscle. This electrical activity is primarily controlled by the cardiac conduction system. ("The heart", 2022). The key functional parameters are systolic and diastolic functions. These are essential for maintaining adequate blood flow and pressure (Trifunovic-Zamaklar *et al,* 2022). Echocardiography and electrocardiograms are essential tools for evaluating cardiac function. These techniques offer valuable insights into heart rate, rhythm, and structural

integrity. (Johnson *et al*, 2022). The heart's structure and function are closely intertwined. However, recent research indicates that the traditional models of cardiac mechanics may require re-evaluation. Novel insights into the heart's dynamic actions are challenging long-standing beliefs about its operation, which could pave the way for innovative approaches in cardiac care.

(Buckberg *et al*, 2018).

1.3.9. Heart valves

The heart's valves, both the atrioventricular (AV) and semilunar, have distinct structural and functional characteristics that are crucial for their roles in the cardiac cycle. The AV valves,

which include the mitral and tricuspid valves, have a complex apparatus involving chordae tendineae and papillary muscles. This apparatus prevents regurgitation (backflow) during ventricular contraction. In contrast, the semilunar valves, such as the aortic and pulmonary valves, are self-supporting structures that close passively due to pressure changes, without the need for chordae tendineae.

1.4. Cardiovascular Diseases overview

Heart disease encompasses a range of conditions related to the heart and blood vessels. The most common form is atherosclerosis, which occurs when plaque accumulates in the artery walls. This plaque buildup narrows the arteries, restricting blood flow. If a blood clot forms, it can completely block blood flow, leading to a heart attack or stroke. (AHA *et al*, 2024) cardiovascular diseases (CVDs) are the leading cause of death globally, claiming an estimated 17.9 million lives each year. CVDs encompass a group of disorders affecting the heart and blood vessels, including coronary heart disease, cerebrovascular disease, rheumatic heart disease, and other conditions. More than four out of five CVD deaths are due to heart attacks and strokes, and one-third of these deaths occur prematurely in people under 70 years of age. The most significant behavioral risk factors for heart disease and stroke are unhealthy diet, physical inactivity, tobacco use, and harmful use of alcohol. Additionally, environmental factors, such as air pollution, play a crucial role. These behavioral risk factors can manifest as intermediate risk factors, including raised blood pressure, blood glucose, blood lipids, and overweight or obesity. These intermediate risk factors can be measured in primary care facilities and indicate an increased risk of heart attack, stroke, heart failure, and other complications. (WHO 2022)

1.4.1 coronary artery diseases

Coronary artery disease (CAD) is a common and serious condition. It is caused by the narrowing of the coronary arteries, which supply blood to the heart. This narrowing is due to atherosclerosis, a process where plaque builds up in the arteries. The plaque is made up of fats, cholesterol, and inflammatory cells. (Anil and cheema *et al*, 2021) (Alwis and Wijesinghe *et al*, 2023). This disease is a major global health concern, causing over 600,000 deaths in the United States each year. (Gopalan and Kirk *et al*, 2022). Coronary artery disease (CAD) is a complex condition that requires a comprehensive understanding. Key aspects to consider include: 1. Risk Factors: - Identify the major risk factors, such as high cholesterol, high blood pressure, diabetes, smoking, and family history. - Understand how these factors contribute to the development and progression of CAD. 2. Pathogenesis: - Explain the underlying mechanisms that lead to the formation and buildup of plaque in the coronary arteries. - Describe the impact of plaque accumulation on the heart's ability to receive adequate blood flow and oxygen. 3. Management Strategies: - Outline the various treatment approaches, including lifestyle modifications, medications, and interventional procedures. - Emphasize the importance of early detection, risk factor management, and a multidisciplinary approach to effectively manage CAD. By addressing these key aspects, you can provide a clear and concise overview of the essential elements involved in understanding coronary artery disease.

1.4.2. Congenital heart defect

Congenital heart defects (CHDs) are structural abnormalities of the heart or great vessels present at birth, making them the most common type of birth defect. These defects can lead to significant health issues, including heart failure and increased mortality rates among infants. The causes of CHDs are multifactorial, involving genetic and environmental influences, and they can manifest in various forms, ranging from mild to life-threatening conditions.

Types of Congenital Heart Defects

Septal Defects: The two most common types of septal defects are: 1. Ventricular Septal Defects (VSD): These are openings or holes in the wall (septum) that separates the two lower chambers of the heart (ventricles). 2. Atrial Septal Defects (ASD): These are openings or holes in the wall (septum) that separates the two upper chambers of the heart atria (Cervantes-Salazar *et al,* 2024). Complex congenital heart defects, such as Tetralogy of Fallot and transposition of the great vessels, are significant medical conditions. Congenital heart defects (CHDs) frequently co-occur with other birth defects, affecting multiple organ systems. (Alymbaev *et al*, 2024).

1.4.3. Arrhythmia

Arrhythmias, or heart rhythm disorders, can have various underlying causes, including: 1. Structural heart issues: Conditions that affect the structure or function of the heart, such as heart disease, can lead to arrhythmias. 2. Lifestyle factors: Certain lifestyle choices, such as excessive alcohol consumption, smoking, or lack of physical activity, can contribute to the development of arrhythmias. 3. Medication effects: Some medications, either prescribed or over-the-counter, can have side effects that disrupt the heart's normal rhythm. Understanding these potential causes are essential for developing effective prevention strategies. By addressing the underlying factors, individuals can take proactive steps to reduce their risk of developing arrhythmias. Common Causes of Arrhythmias Structural Heart Conditions: Congenital (present at birth) abnormalities, heart diseases, and issues with heart valves can disrupt the normal electrical signaling in the heart (Leonova *et al*, 2021) Electrolyte Imbalances: Hypo/hyperkalemia and hypo/hypermagnesemia are significant modifiable risk factors Certain medications can cause heart rhythm disturbances (arrhythmias). These include: Antiarrhythmic drugs - Psychotropic medications (e.g., antidepressants, antipsychotics) - Over-the-counter cold medicines Lifestyle factors that can

trigger migraines include stress, excessive caffeine intake, tobacco use, alcohol consumption, and use of illegal stimulants (Polsdorfer *et al*, 2011).

1.4.4. Dilated cardiomyopathy

Dilated cardiomyopathy (DCM) is a complex heart condition characterized by the enlargement of the heart's ventricles (lower chambers) and reduced contractility (ability to pump blood effectively). Both genetic and acquired factors contribute to the development of DCM. Understanding the underlying causes of this condition is crucial for implementing effective management and treatment strategies.

1.4.5. Myocardial infarction

Myocardial infarction (MI), commonly known as a heart attack, is primarily caused by coronary artery disease (CAD). CAD leads to reduced blood flow and oxygen supply to the heart muscle, resulting in tissue damage. The main underlying causes of MI include: 1. Atherosclerosis: The buildup of plaque in the coronary arteries, which can restrict blood flow. 2. Hypertension (high blood pressure): Puts additional strain on the heart, contributing to the development of CAD 3. Diabetes: Increases the risk of developing atherosclerosis and other cardiovascular complications. 4. Lifestyle factors: Smoking, obesity, and physical inactivity are significant risk factors for MI. These underlying factors not only contribute to the onset of MI but also significantly impact patient outcomes. Early detection and prompt intervention are crucial in managing and preventing the devastating consequences of a heart attack. Key Causes of Myocardial Infarction Coronary Artery Disease (CAD) is the leading cause of cardiovascular disease, characterized by

the buildup of plaque in the coronary arteries, which can lead to ischemia (reduced blood flow). The primary risk factors for CAD include hypertension, diabetes, hyperlipidemia (high cholesterol), smoking, and obesity (Khan *et al*, 2024; Żuber *et al*,2024).

1.4.6 Heart failure

Heart failure (HF) is a complex condition influenced by various factors. The main contributors include medical conditions, lifestyle choices, and demographic factors. Understanding these factors is crucial for effective prevention and management of heart failure. The common causes and risk factors associated with heart failure are as follows: Hypertension, or high blood pressure, is a major risk factor for heart failure. It significantly increases the chances of developing this serious cardiovascular condition. Diabetes Mellitus and Heart Failure: Uncontrolled diabetes, particularly type 2 diabetes, is strongly associated with an increased risk of developing heart failure. Individuals with poorly managed diabetes are more susceptible to experiencing heart failure, a serious condition where the heart is unable to effectively pump blood throughout the body. The link between diabetes and heart failure is well- established. Diabetes can lead to various cardiovascular complications, including damage to the heart muscle, impaired blood vessel function, and increased inflammation. These factors contribute to the development and progression of heart failure in people with uncontrolled diabetes. It is crucial for individuals with diabetes to maintain tight glycemic control, adhere to their treatment regimens, and work closely with their healthcare providers to manage their condition effectively. Proper diabetes management can help reduce the risk of heart failure and other cardiovascular complications, ultimately improving overall health outcomes. (Wang *et al*, 2024) (P, *et al*, 2023). Coronary Artery Disease (CAD) is a significant contributor to the development of heart failure. CAD is

characterized by the buildup of plaque in the coronary arteries, which can restrict blood flow to the heart muscle. This reduced blood flow can lead to ischemic heart disease, a condition where the heart muscle is deprived of oxygen and nutrients, ultimately resulting in heart failure. (Chaudhry et al, 2024) (P *et al*, 2023). Cardiomyopathy: Cardiomyopathy, is a condition that affects the heart muscle, is a significant contributor to heart failure. Among the different types of cardiomyopathies, dilated cardiomyopathy is particularly common.

1.4.7. Cardiovascular Disease risk factor

Cardiovascular disease (CVD) risk is influenced by a combination of genetic and environmental factors. Specific genetic variations, or polymorphisms, can increase an individual's susceptibility to CVD. These genetic predispositions interact with lifestyle choices and environmental exposures, ultimately determining a person's overall CVD risk profile. Understanding these contributing factors is essential for developing effective strategies to prevent and manage cardiovascular disease.

1.4.7. Genetic Factors

Polymorphisms: Genetic variations in the renin-angiotensin-aldosterone system (RAAS), lipid metabolism, and homocysteine regulation significantly contribute to cardiovascular disease (CVD) risk. Key examples include variations in the ACE and ApoE genes, which are associated with cholesterol metabolism and blood pressure regulation. (Antonova and Todorova *et al*, 2023). Heritability: Research suggests that certain traits, like body mass index (BMI) and cholesterol levels, have a strong genetic basis, especially during childhood and adolescence. This indicates that genetics play a significant role in the risk of developing cardiovascular disease (CVD).

1.4.8. Environmental Factors

Lifestyle Factors and Cardiovascular Disease Certain lifestyle choices can significantly contribute to the development of cardiovascular disease (CVD). These include obesity, physical inactivity, smoking, and poor diet. For example, having a high body mass index (BMI) is consistently linked to an increased risk of CVD. (Ballin *et al*, 2023) (Blann *et al*, 2024. Shared Environment: Childhood environmental factors significantly influence the development of cardiometabolic risk factors. ("How Genetic and Environmental Factors Influence Cardiometabolic Risk Factors? Findings from the Isfahan Twins Study", 2023).

1.4.9. Dimethylhydrazine (DMH) as a risk factor for cardiovascular disease.

Dimethylhydrazine (DMH) exposure is associated with an increased risk of cardiovascular disease (CVD) through several mechanisms, primarily involving: I. Oxidative stress: DMH exposure can lead to an imbalance between the production of reactive oxygen species and the body's ability to neutralize them, resulting in cellular damage and inflammation. ii. Endothelial dysfunction: DMH can impair the normal function of the endothelium, the inner lining of blood vessels, leading to reduced vasodilation and increased vascular resistance. iii. Abnormal cellular proliferation: DMH exposure can disrupt the normal growth and division of cells, leading to the development of cardiovascular complications. These mechanisms collectively contribute to the increased risk of cardiovascular complications following DMH exposure. Chronic exposure to DMH has been linked to significant cardiotoxic effects, such as changes in heart mass and impaired myocardial energy metabolism. These alterations can lead to decreased cardiac function and increased risk of cardiovascular diseases over time (Ukolov *et al*, 2022).

1.5. How Dimethylhydrazine (DMH) affects the heart

Dimethylhydrazine (DMH) has been found to have significant negative effects on the heart and cardiovascular system. Studies show that DMH can disrupt the normal function of the heart muscle and the blood vessels. The primary mechanisms behind these effects appear to be metabolic disturbances and the overgrowth of endothelial cells (the cells that line the blood vessels). As a result, DMH exposure can lead to structural and functional changes in the heart. The cardiotoxic effects of DMH are linked to the inhibition of energy metabolism in the heart muscle, which results in reduced contraction and relaxation rates in the left ventricle. (Ukolov *et al*, 2022). While DMH poses risks to cardiac health, some studies suggest potential protective strategies. For example, the use of dimethyl fumarate may mitigate oxidative damage in cardiomyocytes. This highlights the complex interplay between harmful and protective agents in cardiac pathology. (Kuang *et al*, 2020).

CHAPTER TWO

MATERIAL AND METHODS

2.1 EQUIPMENT AND APPARATUS

The equipment and apparatus used in this study were spectrometer, centrifuge, digital weighing balance, Pestle and mortal, Glass pipette, Micro pipette, Cuvette, Syringes, Needles, Tag pins, Cardboard paper, Tissue bags, Rat cages, Test tubes, Test tubes racks, Dissecting sets, Refrigerator, Beakers, Volumetric flask, measuring cylinder, Feeding and drinking troughs, pH meter, Water, baths, Gloves, Freezer, Sample bottles (Heparin, plain, EDTA) and Stirring rod.

CHEMICALS AND REAGENT

The chemical and reagent used in this study were, Greiss reagent, Petroleum, Pyrogallol, Trichloroacetic acid, Thiobarbituric acid, Hydrochloric acid, Adrenaline, Potassium permanganate solution (km lo4), Potassium Dihydrogen phosphate, Disodium Hydrogen phosphate, Phosphate buffer hydrogen Peroxide, Plasma Vitamin E, Plasma Vitamin A, EDTA, Anhydrous Sodium Hydrogen Carbonate, Sodium hydrogen carbonate, Chloroform, Digitonin, Xylene, 2,4-dinitrophenyl hydrazine (DNPH) Reagent, Absolute ethanol , Distilled water, Normal saline, Dipyridyl, Nitric oxide, Sodium citrate , Ferric chloride, Alpha-Alpha-dipyridyl, Nitroprusside.

2.2 Methodology

2.2.1 Collection and preparation of plant materials

Preparation and Extraction of *Vernonia amygdalina*

Mature leaves of the Vernonia amygdalina plant were collected from Ekosodin road in Benin City, Edo State. The leaves were separated from the stalks, washed, and air-dried at room temperature (24°C). The dried leaves were then pulverized into a fine powder and weighed. To prepare the ethanol extract, 400g of the powdered leaves were soaked in 1000ml of absolute ethanol at room temperature for 72 hours, with intermittent stirring every 4 hours. The mixture was then filtered using muslin cloth. The filtered extract was concentrated using a rotary evaporator at 40°C, reducing it to one-tenth of its original volume. The concentrated extract was then freeze-dried to obtain the crude extract, which was stored in a refrigerator.

2.2.2 Experimental animals

Thirty-five male albino Wistar rats were obtained from the Department of Pharmacognosy at the University of Benin. The rats were housed in the animal facility of the Biochemistry Department at the same university. After a two-week acclimation period, the animals were fed a grower pellet diet and provided with clean drinking water for the duration of the experiment.

2.2.3 Experimental design

The experiment used 35 rats, which were divided into 8 groups. Group 1 (normal control) received regular food and water, but no Vernonia amygdalin extract, silymarin, antibiotics, or 1,2'-dimethyl hydrazine. Group 2 (1,2-dimethylhydrazine (DMH) control) received DMH along with food and water to induce liver damage. Group 3 received Silymarin, a known antibiotic, after being induced with DMH (a chemical compound).

Group	1 (Normal control)	2 (DMH only)	3 (Silymarin Control)	4 (Extract only)	5 (Post Treatment 1)	6 (Post Treatment 2)	7 (Pre Treatment1)	8 (Pre Treatment2)
Standard feed	Administered	Administered	Administered	Administered	Administered	Administered	Administered	Administered
Water	Administered	Administered	Administered	Administered	Administered	Administered	Administered	Administered
DMH		Administered						
Silymarin			Administered					
Plant extract				Administered	Administered	Administered	Administered	Administered

2.2.4 Induction of 1,2-dimethyl hydrazine

To induce liver damage, 1,2-dimethyl hydrazine was administered orally by gavage at a dose of 3 mL/kg body weight. Within a few days, the animals exhibited reddish urine, loss of appetite, and weight loss. Immediately, and for the following 21 days, silymarin antibiotics (100 mL/kg body weight) and Vernonia amygdalina extract were administered daily.

2.2.5. Animal Sacrifice

The rats were euthanized using chloroform as an anesthetic. After dissection, blood samples were collected from the aorta and stored in labeled EDTA tubes. The blood plasma was separated by centrifugation at 4000 rpm for 10 minutes. The following organs were collected, weighed, and placed in marked plastic bags in an ice-filled bucket: kidney, heart, pancreas, colon, and liver.

2.2.6. Preparation of liver serum

A 1-gram sample of liver tissue was excised and mixed with 5 milliliters of normal saline solution. The mixture was then centrifuged at 4,000 revolutions per minute for 20 minutes. The clear supernatant, or serum, was carefully aspirated and transferred into plain storage bottles. These bottles were stored at -4 degrees Celsius in a refrigerator until needed for biochemical analysis.

2.2.7. Biochemical Assays

Liver function tests were conducted on rats from all 8 groups. The following parameters were quantitatively analyzed: 1. Total protein 2. Total cholesterol 3. Triacylglycerol 4. High-density lipoprotein cholesterol 5. Very low-density lipoprotein cholesterol 6. Low-density lipoprotein cholesterol 7. Atherogenic index of plasma 8. Atherogenic coefficient 9. Nitric Oxide 10. Cardiac risk ratio.

2.2.8 ESTIMATION OF TOTAL PROTEIN

Principle

In alkaline conditions, cupric ions (Cu2+) interact with the peptide bonds of proteins, forming a colored complex.

Composition of Reagents

Reagent 1 (Biuret Reagent): - 100 mmol/L Sodium hydroxide - 16 mmol/L Sodium-Potassium tartrate - 15 mmol/L Potassium iodide - 6 mmol/L Copper sulfate Reagent

2. (Blank Reagent): - 100 mmol/L Sodium hydroxide - 16 mmol/L Sodium-Potassium tartrate Procedure: 1. Pipette 100 µL of sample, standard, and distilled water into labeled sample, standard, and blank test tubes, respectively. 2. Add 1000 µL of Reagent 1 to all tubes.

3. Incubate the tubes for 30 minutes at 25°C. 4. Measure the absorbance of the sample at 546 nm against the reagent blank.

Calculation:

Formula for concentration of total Protein (Mg/ml)

Total protein concentration=190 ×A sample

2.2.9 ESTIMATION OF NITRIC OXIDE

Principle

Sodium nitroprusside, when dissolved in an aqueous solution at pH 7.2, produces nitric oxide. This nitric oxide then reacts with oxygen to form nitrite ions. The concentration of these nitrite

ions can be measured using the Griess reagent, which generates a pink color. The absorbance of this color can be measured at 540 nm. Procedure:

1. Pipette 0.5 ml of serum into labeled tubes.

2. Dispense 0.5 ml of sodium nitroprusside, which was prepared in 10 mM of potassium phosphate buffer (pH 7.4), into all tubes.

3. Incubate the tubes for 15 minutes at room temperature (25°C).

4. Read the initial absorbance.

5. Add 1.0 ml of Griess reagent, which contains an equal volume of solution A (2% sulfanilamide and 4% H3PO4) and solution B (0.2 ml naphthyl ethylenediamine dihydrochloride), to all tubes.
6. Read the absorbance of the chromophore formed at 540 nm.

Calculation:

A0- A1\ A0 X 100

A0= Absorbance before reaction

A1= Absorbance after reaction with greiss reagent

2.2.10 Estimation of total cholesterol

The total cholesterol level is determined through an enzymatic reaction. This reaction converts cholesterol into a colored compound, which is then measured using a spectrophotometer.

Principle:

The cholesterol assay uses enzymes, such as cholesterol oxidase and cholesterol esterase, to break down cholesterol into cholesterol oxide. This cholesterol oxide then reacts with a

chromogen, like 4-aminoantipyrine, to form a colored compound called quinoneimine. The intensity of the color is directly proportional to the concentration of cholesterol in the sample.

Procedure:

- Sample Preparation: A serum or plasma sample is obtained from the patient and mixed with a reagent containing enzymes and a chromogen.
- Enzymatic Reaction: The mixture is incubated at 37°C for 5-10 minutes, allowing the enzymatic reaction to occur.
- Color Development: The reaction between cholesterol oxidase and the chromogen produces a colored compound, quinonimine.
- Measurement: The absorbance of the colored compound is measured spectrophotometrically at 500-550 nm.

Reagent 1: Cholesterol Oxidase Reagent - Cholesterol oxidase (5-10 U/L) - Cholesterol esterase (2-5 U/L) - 4-Aminoantipyrine (0.5-1.0 mmol/L) - Phenol (1.0-2.0 mmol/L) - Sodium phosphate buffer (50-100 mmol/L, pH 7.0-7.5) Reagent 2: Chromogen Reagent - 4-Aminoantipyrine (0.5-1.0 mmol/L) - Phenol (1.0-2.0 mmol/L) - Sodium phosphate buffer (50-100 mmol/L, pH 7.0-7.5) Reagent 3: Standard/Calibrator - Cholesterol standard (200-400 mg/dL) - Sodium cholate or other stabilizers Reagent 4: Control - Cholesterol control material (normal and abnormal levels).

Calculation:

Total Cholesterol (mg/dl) = (O.D of Sample) \ (O.D of standard) x Concentration of standard.

2.2.11 Estimation of Triacylglycerol

Principle

The triacylglycerol (TAG) assay uses an enzymatic reaction to convert TAG into glycerol, which is then measured spectrophotometrically.

Reagent Composition: -

Reagent 1 (Lipase Reagent): - Lipase (5-10 U/L) - Glycerol kinase (2-5 U/L) - Glycerol-3-phosphate oxidase (1-2 U/L) - Peroxidase (1-2 U/L) - 4-Aminoantipyrine (0.5-1.0 mmol/L) - Phenol (1.0-2.0 mmol/L) - Sodium phosphate buffer (50-100 mmol/L, pH 7.0-7.5) - Reagent 2 (Chromogen Reagent): - 4-Aminoantipyrine (0.5-1.0 mmol/L) - Phenol (1.0-2.0 mmol/L) - Sodium phosphate buffer (50-100 mmol/L, pH 7.0-7.5) - Reagent 3 (Standard/Calibrator): - Triacylglycerol standard (100-400 mg/dL) - Sodium cholate or other stabilizers - Reagent 4 (Control): - Triacylglycerol control material (normal and abnormal levels)

Procedure:

1. Collect a serum or plasma sample from the patient. 2. Prepare the lipase reagent and chromogen reagent as directed by the manufacturer. 3. Combine the patient sample with the lipase reagent and incubate at 37°C for 5-10 minutes. 4. Add the chromogen reagent to the reaction mixture and incubate for an additional 5-10 minutes. 5. Measure the absorbance.

Calculation:

Concentration (g\l) = Mass of TAG (g) \ Volume of solution (l)

2.2.12 High-Density Lipoprotein (HDL) Cholesterol Estimation

HDL cholesterol is a key indicator of cardiovascular disease risk. There are several methods to measure HDL cholesterol, but the most commonly used are precipitation-based methods and homogeneous (direct) assays.

1. Principle

The HDL cholesterol assay typically involves two main steps: 1. Separation of HDL particles from other lipoproteins: This step selectively removes or blocks the non-HDL lipoproteins, such as low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL), allowing for the isolation of HDL particles. 2. Quantification of HDL cholesterol: After the separation step, the concentration of cholesterol specifically associated with the HDL particles is measured using various analytical techniques, such as enzymatic colorimetric methods or direct HDL cholesterol assays. The goal of the HDL cholesterol assay is to accurately determine the level of HDL cholesterol, which is an important biomarker for cardiovascular health. By isolating the HDL particles and quantifying the cholesterol content, healthcare professionals can assess an individual's risk of developing heart disease and make appropriate treatment or lifestyle recommendations.

Reagent Compositions

Precipitation Method Reagents: 1. Precipitation Reagent: - Phosphotungstic acid (0.55 mmol/L) or Dextran sulfate (50,000Da) - Magnesium chloride (25 mmol/L) Cholesterol Assay Reagents: 1. Cholesterol esterase: Hydrolyzes cholesterol esters to free cholesterol. 2. Cholesterol oxidase: Converts free cholesterol to cholestenone and hydrogen peroxide. 3. Peroxidase (POD): Reacts with hydrogen peroxide to form a colored complex. 4. Chromogen (e.g., 4-Aminoantipyrine & Phenol): Participates in the color formation reaction.

Procedure

Precipitation Method (Traditional) 1. Precipitation of LDL and VLDL (a) Mix 200 µL of serum with 500 µL of precipitation reagent. (b). Incubate at room temperature for 10-15 minutes. (c). Centrifuge at 3000 rpm for 10 minutes. d. Collect the clear supernatant, which contains HDL.

2. Enzymatic Cholesterol Estimation

Add 1 mL of cholesterol reagent to 50 µL of supernatant. 2. Incubate the mixture at 37°C for 5-10 minutes. 3. Measure the absorbance of the sample at 500 nm using a spectrophotometer. 4. Use a cholesterol standard to calculate the HDL concentration.

Calculation:

HDL (mg\dl) = (Abs Sample) \ (Abs Standard) x Concentration of standard

Abs of Sample = Absorbance of the test sample

Abs of Sample = Absorbance of the Cholesterol standard.

Concentration of standard =200mg\dL

2.2.13 Atherogenic Index of Plasma (AIP)

The Atherogenic Index of Plasma (AIP) is a lipid-based biomarker used to assess the risk of cardiovascular disease (CVD). It is calculated by taking the logarithm of the ratio between triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C).

Principle

The AIP (Atherogenic Index of Plasma) is a calculated value derived from the lipid profile. It is expressed as: AIP = log(triglycerides/HDL-cholesterol) This concise formulation clearly states

what the AIP is, where it comes from (the lipid profile), and how it is calculated. The key information is presented in a straightforward manner without unnecessary details.

Reagent Composition

The Atherogenic Index of Plasma (AIP) requires the measurement of triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C) using enzymatic assays. A. Triglyceride (TG) Measurement: 1. Lipoprotein lipase hydrolyzes triglycerides into glycerol and free fatty acids. 2. Glycerol kinase converts glycerol into glycerol-3-phosphate. 3. Glycerol-3-phosphate oxidase produces hydrogen peroxide. 4. Peroxidase and a chromogen (e.g., 4-Aminoantipyrine + Phenol) form a colored complex that is measured at 500 nm. B. HDL-Cholesterol (HDL-C) Measurement: 1. Precipitation Reagent (for traditional HDL measurement): - Phosphotungstic acid or Dextran sulfate selectively precipitates LDL and VLDL. - Magnesium chloride ($MgCl_2$) helps in the precipitation. 2. Cholesterol Enzymes for HDL Estimation: - Cholesterol esterase converts HDL cholesterol esters into free cholesterol. - Cholesterol oxidase produces hydrogen peroxide. - Peroxidase and a chromogen are used for color formation.

Calculation:

ALP = Log (TG) \ (HDL-C)

TG= Triglycerides (mg\dL)

HDL-C (Mg\dL)

2.2.14 Atherogenic Coefficient

Principle

The Atherogenic Coefficient (AC) is a measure used to assess cardiovascular risk. It evaluates the balance between atherogenic lipoproteins (LDL and VLDL) and anti-atherogenic lipoproteins (HDL). This ratio provides insight into the overall lipid profile and can be used to determine an individual's risk of developing cardiovascular disease.

Reagent Composition

Enzymatic colorimetric assays are used to measure Total Cholesterol (TC) and HDL-Cholesterol (HDL-C). For TC measurement: 1. Cholesterol esterase hydrolyzes cholesterol esters to free cholesterol. 2. Cholesterol oxidase converts free cholesterol into cholestenone and hydrogen peroxide (H_2O_2). 3. Peroxidase (POD) and a chromogen (e.g., 4-Aminoantipyrine + Phenol) react with H_2O_2 to form a colored complex, which is measured at 500 nm. For HDL-C measurement: 1. The precipitation method uses a reagent containing phosphotungstic acid or dextran sulfate, which precipitates LDL and VLDL. Magnesium chloride ($MgCl_2$) aids in the precipitation. 2. The cholesterol enzyme reagents used are the same as those for TC measurement.

Calculation:

AC= Total Cholesterol- HDL-C\ HDL-C

Total Cholesterol (TC) includes all Lipoproteins (LDL, VLDL, and HDL)

HDL-C is protective and helps remove excess cholesterol

A higher AC value indicates a higher risk of atherosclerosis.

2.2.15 Cardiac Risk Ratio

Principle

The Cardiac Risk Ratio (CRR), also known as the Total Cholesterol/HDL Ratio, is a measure used to assess the risk of developing cardiovascular disease (CVD). It provides insight into the balance between atherogenic lipoproteins (LDL and VLDL) and anti-atherogenic lipoproteins (HDL). This ratio helps healthcare professionals evaluate an individual's risk of CVD based on their cholesterol profile.

Total Cholesterol (TC) is composed of three main types of cholesterol: LDL, VLDL, and HDL. HDL-Cholesterol (HDL-C) is the "good" cholesterol that provides protective benefits. A higher Coronary Risk Ratio (CRR) value indicates an increased risk of developing atherosclerosis and cardiovascular disease.

Reagent Composition

The Cholesterol Ratio (CRR) calculation requires measuring Total Cholesterol (TC) and HDL-Cholesterol (HDL-C) using enzymatic colorimetric assays. A. Total Cholesterol (TC) Measurement: 1. Cholesterol esterase hydrolyzes cholesterol esters into free cholesterol. 2. Cholesterol oxidase converts free cholesterol into cholestenone and hydrogen peroxide (H_2O_2). 3. Peroxidase (POD) and a chromogen (e.g., 4-Aminoantipyrine + Phenol) react with H_2O_2 to form a colored complex, which is measured at 500 nm. 4. A phosphate buffer (pH 7.0-7.5) maintains reaction stability. B. HDL-Cholesterol (HDL-C) Measurement: 1. Precipitation Method (Traditional Measurement): - Phosphotungstic acid or Dextran sulfate precipitates LDL and VLDL. - Magnesium chloride ($MgCl_2$) enhances the precipitation. - Centrifugation removes the precipitated LDL and VLDL, leaving HDL in the supernatant for measurement. 2. Homogeneous (Direct) HDL Measurement (Modern Method): - Specific detergents and enzymes selectively react with HDL. - No precipitation step is needed. - The reaction is measured directly at 600 nm.

Calculation:

CRR = Total Cholesterol (TC)\ HDL- Cholesterol (HDL-C)

HDL-Cholesterol (HDL-C) is the protective good cholesterol

A higher CRR value indicates an increased risk of Atherosclerosis and Cardiovascular disease.

Statistical Analysis

The data obtained in this study are expressed as mean ± standard error of mean (SEM). The data were analyzed using one way analysis of variance and Duncan Multiple Range Test (SPSS version 21).

CHAPTER THREE

3.0 RESULTS

3.1 Effect of *V. amygdalina* on Cardiovascular Disease Risk Factors

Exposure of normal Wistar rats to a single intraperitoneal dose of DMH significantly elevated their total protein (TP), lipid profile [total cholesterol (TC), triacylglycerol (TG), very-low density lipoprotein cholesterol (VLDL-C), low-density lipoprotein cholesterol (LDL-C)], and cardiovascular disease risk factors [atherogenic index of plasma (AIP), atherogenic coefficient (AC), cardiac risk ratio (CRR)], but these parameters were markedly reduced after treatment with ethanol extract of the medicinal plant ($p < 0.05$). In addition, the extract significantly elevated high-density lipoprotein cholesterol (HDL-C) level which was reduced by DMH ($p < 0.05$). These results are presented in Tables 3.1 – 3.4.

Table 3.1: Lipid Profile of Rats

Group	Hepatic TC (mg/dL)	Plasma TC (mg/dL)	HDL-C (mg/dL)
Control	107.65 ± 1.38	26.88 ± 3.30	54.38 ± 1.68
DMH	214.05 ± 3.95	92.75 ± 0.44	16.01 ± 0.98
Silymarin	168.70 ± 13.80	47.83 ± 1.68	47.93 ± 0.89
Extract Only	176.85 ± 2.45	42.62 ± 0.18	51.11 ± 2.48
Post-treatment 1	128.05 ± 2.65	57.03 ± 1.51	55.70 ± 2.12
Post-treatment 2	148.30 ± 33.70	52.52 ± 0.18	49.51 ± 5.66

N = 5

Table 3.2: Levels of Total Protein and Tissue/Plasma Triacylglycerol

Group	Hepatic TG (mg/dL)	**Plasma TG (mg/dL)**	**TP (g/dL)**
Control	21.49 ± 1.18	42.52 ± 2.01	1.50 ± 0.17
DMH	42.01 ± 0.40	93.61 ± 4.56	8.41 ± 0.13
Silymarin	32.25 ± 1.48	48.54 ± 1.83	2.75 ± 0.90
Extract Only	29.00 ± 0.42	49.09 ± 0.92	2.41 ± 0.43
Post-treatment 1	29.79 ± 1.94	62.41 ± 1.10	2.95 ± 0.35
Post-treatment 2	27.59 ± 1.01	45.99 ± 17.89	4.07 ± 0.04

N = 5

Table 3.3: Levels of Cardiac Nitric Oxide and Very-low/Low Lipoprotein Cholesterol

Group	**NO (mmol/L)**	**Hepatic VLDL-C (mg/dL)**	**Plasma VLDL-C (mg/dL**	**LDL-C (mg/dL)**
Control	36.95 ± 12.55	4.30 ± 0.58	8.50 ± 1.04	44.77 ± 4.02
DMH	59.95 ± 2.05	8.40 ± 0.91	18.72 ± 1.53	179.32 ± 7.53
Silymarin	30.10 ± 5.40	6.45 ± 0.68	9.71 ± 1.09	111.06 ± 3.84
Extract Only	41.80 ± 0.60	8.36 ± 0.42	9.82 ± 0.88	115.92 ± 4.11
Post-treatment 1	41.50 ± 0.80	5.80 ± 0.54	12.48 ± 0.93	59.87 ± 2.58
Post-treatment 2	40.85 ± 1.85	5.52 ± 0.51	9.20 ± 1.27	89.59 ± 2.82

N = 5

Table 3.3: Cardiovascular Disease Risk Factors

Group	AIP	AC	CRR
Control	44.93 ± 2.37	0.98 ± 0.06	1.98 ± 0.08
DMH	180.49 ± 8.26	12.37 ± 1.16	13.37 ± 0.85
Silymarin	111.26 ± 5.84	2.52 ± 0.46	3.52 ± 0.28
Extract Only	116.11 ± 5.03	2.46 ± 0.18	3.46 ± 0.56
Post-treatment 1	60.09 ± 1.62	1.30 ± 0.09	2.30 ± 0.40
Post-treatment 2	89.78 ± 2.84	2.00 ± 0.21	3.00 ± 0.50

N = 5

CHAPTER FOUR

DISCUSSION AND CONCLUSION

4.1 Discussion

Toxicity is the degree to which a chemical substance or a specific mixture of substances can cause harm to an organism (Laios *et al*. 2021). Dangerous chemicals possess physical toxicity because they destroy tissues, but are not directly poisonous unless they interfere directly with biological activity. Toxicity can occur through various routes of exposure (ingestion, inhalation, skin contact, or injection), thereby resulting in chemical reactions with biological molecules, disruption of cellular processes, DNA damage and mutations, and oxidative stress and inflammation (Laios *et al*. 2021). 1, 2- Dimethylhydrazine (DMH), is a potent toxicant, inducing colorectal tumors in experimental animals. It is the most widely used model of chemically induced colon carcinogenesis (Perse and Cerar, 2011). The main route of administration of DMH is subcutaneous injection, but intraperitoneal injections also succeed to produce tumors in the colon (Ghadi *et al*., 2013). The first oxidation step of DMH involves its oxidation to azomethane, which is converted into azoxymethane (AOM) and then hydroxylated to methylazoxymethanol (MAM) (Karthikkumar *et al*., 2020). Hydroxylation occurs predominantly in the liver, probably via cytochrome P450-dependent pathway and to a limited degree in the colonic mucosa (Weisburger *et al.*, 1971; Karthikkumar *et al.,* 2020).

Traditional/herbal medicine is complimentarily being used in many areas of the world (especially developing countries) for health care management. Of late, the interest in plant products surfaces all over the world due to the belief that many herbal medicines are known to be free from side effects. It is the fact that the discovery of new synthetic drug is time consuming and an expensive

affair. The utility of the synthetic drug is always accompanied with its single or multiple adverse effects (George *et al.*, 2011). Herbs had been used by all cultures throughout history. At present, the demand for herbal products is growing exponentially throughout the world and major pharmaceutical companies are currently conducting extensive research on plant materials for their potential medicinal value (Islam *et al.,* 2021). *Vernonia amygdalina*, a member of the daisy family, is a small shrub that grows in tropical Africa (Toyang and Verpoorte *et al.*, 2013). Popularly known as bitter leaf, the medicinal plant is widely used in traditional medicine across various parts of Nigeria (Farombi and Owoeye *et al.*, 2011). The leaves of this plant contain numerous bioactive compounds which offers a wide range of potential health benefits. Its biologically-active compounds such as polyphenols are good antioxidants which can reduce oxidative stress caused by the accumulation of free radicals (Rohin *et al.,* 2016). Chemicals ingested by animals always end up in the liver where they are metabolized. The products of their modifications have far-reaching effect on other organs and the systemic circulation. Whereas DMH is a colon carcinogen, its products of metabolism impact other organs negatively, including the heart.

In this study, the effect of ethanol extract of *V. amygdalina* leaves on cardiovascular disease risk factors in rats exposed to DMH was investigated. The results indicated that exposure of normal Wistar rats to a single intraperitoneal dose of DMH significantly elevated their total protein, lipid profile, and cardiovascular disease risk factors, but these parameters were markedly reduced after treatment with ethanol extract of the medicinal plant. In addition, the extract significantly elevated high-density lipoprotein cholesterol (HDL-C) level which was reduced by DMH. These results suggest that the toxicant DMH has profound toxic effect and functioning capacity of the heart, and that *V. amygdalina* leaves extract can significantly ameliorate the

dangerous/deleterious effect of the compound on the heart by improving cardiac total protein, lipid profile, nitric oxide level and the cardiovascular disease risk factors. Lipid profile is a panel of blood tests that serves as an initial broad medical screening tool for the assessment of abnormalities in the concentrations of lipids, such as cholesterol and triacylglycerol. These tests can identify certain genetic diseases and determine approximate risks for cardiovascular diseases (CVDs), certain forms of pancreatitis, and other diseases. Lipid profile typically includes LDL-C, HDL-C, TG, TC, VLDL-C and CRR (Reiser *et al.,* 1985). Abnormal lipid profile is seen in those with severe liver dysfunction (Halsted, 2004). There is a prominent decline in plasma TC and TG levels in patients with severe hepatitis and hepatic failure because of reduction in lipoprotein biosynthesis (Halsted, 2004). The results obtained in this study suggest that the lipids synthesis capacity of the liver may be reduced with DMH induction, and are in agreement with those of previous reports (Sposito *et al.,* 1997; Selimoglu *et al.,* 2002; Siagris *et al.,* 2006). It is likely the ethanol extract of *V. amygdalina* leaves regulated liver secretion and uptake of plasma lipoproteins. The capacity of the extract to promote lipids biosynthesis could be due to the enhanced transport of acetate into liver cells, resulting in increased substrate (acetate) availability. Elevated levels of serum TC and TG in DMH exposed rats has been reported (Boll *et al.,* 2001; Venkatanarayana *et al.,* 2013). In this study, the effect produced by the extract of the medicinal plant was comparable to that of silymarin (standard cardioprotective drug). Silymarin protects organs against xenobiotic injury by controlling liver secretion and uptake of plasma lipoprotein, while increasing the intracellular glutathione content (Toklu *et al.,* 2008). Silymarin plays the role of an anti-inflammatory agent, through its capacity to inhibit neutrophil infiltration and regulate the release of inflammatory mediators. It has been reported that silymarin prevents chemical-induced lipid peroxidation and toxicity in mice, first, by decreasing the metabolic

activation of the chemical agent and second, by acting as a chain-breaking antioxidant (Letteron *et al.,* 1990). In addition, silymarin is able to stimulate protein synthesis resulting in production of new cardiac cells to replace older and damaged ones (Cecilia *et al*., 2009).

4.2 Conclusion

The toxic perturbation induced by DMH on rat cardiovascular system was significantly blocked by treatment with ethanol extract of *V. amygdalina* leaves. The extent of improvement in lipid profile and cardiovascular disease risk factors of the rats may not be unconnected with the dose used, period of administration, as well as the abundant phytochemicals in the plant.

REFERENCES

Boll, M., Weber, L.W., Becker, E. and Stampfl, A (2001). Hepatocyte damage induced by carbon tetrachloride inhibited lipoprotein secretion and changed lipoprotein composition. *Naturforsch*. 56 (3):283 - 290

Cecilia, L.B., Enrique, J.S., Aldo, D.M. and Marcelo, G.R. (2009). Differential effects of silymarin and its activecomponent silibinin on plasma membrane stabilityand hepatocellular lysis. *Chemico-Biological Interactions*. 179: 297 - 303.

Farombi, E.O. and Owoeye, O. (2011). Bitter leaf. *International Journal of Environmental Research and Public Health,* 8(6): 2533–2555.

George, P. (2011). Concerns regarding the safety and toxicity of medicinal plants - An overview. *Journal of Applied Pharmaceutical Science,* 6: 40–44.

Halsted, C.H. (2004). Nutrition and alcoholic liverdisease. Semin Liver Dis. 24 (3): 289 - 304.

Islam, B.U., Suhail, M., Khan, M.K., Zughaibi, T.A., Alserihi, R.F., Zaidi, S.K. and Tabrez, S. (2021). Polyphenols as anticancer agents: Toxicological concern to healthy cells. *Phytotherapy Research,* 35: 6063–6079.

Karthikkumar, V., Ramachandran, V., Mariadoss, A.V.A., Nurulfiza, M. I. and Rajasekar, P. (2020). Biochemical and molecular aspects of 1, 2-dimethylhydrazine (DMH)-induced colon carcinogenesis: a review. *Toxicology Research*, 9(1): 2–18.

Laios, K., Michaleas, S.N., Tsoucalas, G., Papalampros, A. and Androutsos, G. (2021). The ancient Greek roots of the term Toxic. *Toxicity Reports*, 8: 977 – 979.

Letteron, P., Labbe, G., Degott, C., Berson, A., Fromenty,B., Delaforge, M., Larrey, D. and Pessayre, D. (1990). Mechanism for the protective effects of silymarinagainst carbon tetrachloride-induced lipid peroxida-tion and hepatotoxicity in mice: Evidence that silymarin acts as an inhibitor of metabolic activationand as a chain-breaking antioxidant. *BiochemicalPharmacology*. 39 (12): 2027 - 2034.

Reiser, R., Probstfield, J.L., Silvers, A., Scott,L.W., Shorney, M.L., Wood, R.D., O'Brien, B.C., Gotto,A.M. and Insull, W. (1985). Plasma lipid andlipoprotein response of humans to beef fat, coconut oil and safflower oil. *Am J Clin Nutr*. 42 (2):190 - 197.

Selimoglu, M.A., Aydogdu, S. and Yagci, R.V. (2002). Lipid parameters in childhood cirrhosis and chronicliver disease. *Pediatr Int*. 44 (4): 400 – 403.

Siagris, D., Christofidou, M., Theocharis, G.J., Pagoni, N.,Papadimitriou, C., Lekkou, A., Thomopoulos, K.,Starakis, I., Tsamandas, A.C. and Labropoulou-Karatza,C. (2006). Serum lipid pattern in chronic hepatitis C: histological and virological correlations. *J Viral Hepat.* 13 (1): 56 – 61.19.

Sposito, A.C., Vinagre, C.G., Pandullo, F.L., Mies, S., Raia,S. and Ramires, J.A. (1997). Apolipoprotein and lipidabnormalities in chronic liver failure. *Braz J Med Biol Res*. 30 (11): 1287 – 1290

Toklu, H.Z., Akbay, T., Velioglu-Ogunc, A., Ercan, F.N., Keyer-Uysal, M. and Sener, G. (2008). Silymarin, theGedik, antioxidant component of Silybum marianum,prevents sepsis-induced acute lung and brain injury. *Journal of Surgery Research*. 145 (2): 214 - 222.

Venkatanarayana, G., Sudhakara, G., Rajeswaramma,K. and Indira, P. (2013). Combined effect of curcuminand vitamin E against CCl4-induced liver injury in rats. *American Journal of Life Sciences*. 1 (3): 117 - 124.

APPENDIX

Statistical Analysis

TC LIVER

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	4	1.0765E2	2.76345	1.38173	103.2527	112.0473	103.60	109.80
2	2	2.1405E2	5.58614	3.95000	163.8605	264.2395	210.10	218.00
3	2	1.6870E2	19.51615	13.80000	-6.6456	344.0456	154.90	182.50
4	2	1.7685E2	3.46482	2.45000	145.7198	207.9802	174.40	179.30
5	2	1.2805E2	3.74767	2.65000	94.3786	161.7214	125.40	130.70
6	2	1.4830E2	47.65900	33.70000	-279.8991	576.4991	114.60	182.00
7	3	1.3113E2	17.72465	10.23333	87.1029	175.1638	119.20	151.50
8	2	1.3140E2	5.23259	3.70000	84.3870	178.4130	127.70	135.10
Total	19	1.4519E2	35.90008	8.23604	127.8914	162.4980	103.60	218.00

Multiple Comparisons

Dependent Variable: VAR00002

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1	2	-106.40000*	15.19898	.000	-139.8527	-72.9473
		3	-61.05000*	15.19898	.002	-94.5027	-27.5973
		4	-69.20000*	15.19898	.001	-102.6527	-35.7473
		5	-20.40000	15.19898	.207	-53.8527	13.0527
		6	-40.65000*	15.19898	.022	-74.1027	-7.1973
		7	-23.48333	13.40424	.108	-52.9859	6.0192
		8	-23.75000	15.19898	.146	-57.2027	9.7027
	2	1	106.40000*	15.19898	.000	72.9473	139.8527
		3	45.35000*	17.55027	.025	6.7221	83.9779
		4	37.20000	17.55027	.058	-1.4279	75.8279

	5	86.00000*	17.55027	.000	47.3721	124.6279
	6	65.75000*	17.55027	.003	27.1221	104.3779
	7	82.91667*	16.02113	.000	47.6544	118.1789
	8	82.65000*	17.55027	.001	44.0221	121.2779
3	1	61.05000*	15.19898	.002	27.5973	94.5027
	2	-45.35000*	17.55027	.025	-83.9779	-6.7221
	4	-8.15000	17.55027	.651	-46.7779	30.4779
	5	40.65000*	17.55027	.041	2.0221	79.2779
	6	20.40000	17.55027	.270	-18.2279	59.0279
	7	37.56667*	16.02113	.039	2.3044	72.8289
	8	37.30000	17.55027	.057	-1.3279	75.9279
4	1	69.20000*	15.19898	.001	35.7473	102.6527
	2	-37.20000	17.55027	.058	-75.8279	1.4279
	3	8.15000	17.55027	.651	-30.4779	46.7779
	5	48.80000*	17.55027	.018	10.1721	87.4279

	6	28.55000	17.55027	.132	-10.0779	67.1779
	7	45.71667*	16.02113	.016	10.4544	80.9789
	8	45.45000*	17.55027	.025	6.8221	84.0779
5	1	20.40000	15.19898	.207	-13.0527	53.8527
	2	-86.00000*	17.55027	.000	-124.6279	-47.3721
	3	-40.65000*	17.55027	.041	-79.2779	-2.0221
	4	-48.80000*	17.55027	.018	-87.4279	-10.1721
	6	-20.25000	17.55027	.273	-58.8779	18.3779
	7	-3.08333	16.02113	.851	-38.3456	32.1789
	8	-3.35000	17.55027	.852	-41.9779	35.2779
6	1	40.65000*	15.19898	.022	7.1973	74.1027
	2	-65.75000*	17.55027	.003	-104.3779	-27.1221
	3	-20.40000	17.55027	.270	-59.0279	18.2279
	4	-28.55000	17.55027	.132	-67.1779	10.0779
	5	20.25000	17.55027	.273	-18.3779	58.8779

	7	17.16667	16.02113	.307	-18.0956	52.4289
	8	16.90000	17.55027	.356	-21.7279	55.5279
7	1	23.48333	13.40424	.108	-6.0192	52.9859
	2	-82.91667*	16.02113	.000	-118.1789	-47.6544
	3	-37.56667*	16.02113	.039	-72.8289	-2.3044
	4	-45.71667*	16.02113	.016	-80.9789	-10.4544
	5	3.08333	16.02113	.851	-32.1789	38.3456
	6	-17.16667	16.02113	.307	-52.4289	18.0956
	8	-.26667	16.02113	.987	-35.5289	34.9956
8	1	23.75000	15.19898	.146	-9.7027	57.2027
	2	-82.65000*	17.55027	.001	-121.2779	-44.0221
	3	-37.30000	17.55027	.057	-75.9279	1.3279
	4	-45.45000*	17.55027	.025	-84.0779	-6.8221
	5	3.35000	17.55027	.852	-35.2779	41.9779
	6	-16.90000	17.55027	.356	-55.5279	21.7279

7	.26667	16.02113	.987	-34.9956	35.5289

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

TC PLASMA

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	4	26.8775	6.59198	3.29599	16.3882	37.3668	20.34	34.66
2	2	92.7500	.62225	.44000	87.1593	98.3407	92.31	93.19
3	2	47.8300	2.37588	1.68000	26.4836	69.1764	46.15	49.51
4	2	42.6150	.24749	.17500	40.3914	44.8386	42.44	42.79
5	2	57.0250	2.12839	1.50500	37.9022	76.1478	55.52	58.53
6	2	52.5200	.25456	.18000	50.2329	54.8071	52.34	52.70

7	2	54.8200	2.99813	2.12000	27.8828	81.7572	52.70	56.94
8	2	47.9200	5.24673	3.71000	.7800	95.0600	44.21	51.63
Total	18	49.9150	19.18360	4.52162	40.3752	59.4548	20.34	93.19

Multiple Comparisons

Dependent Variable: VAR00002

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1	2	-65.87250*	3.64932	.000	-74.0037	-57.7413
		3	-20.95250*	3.64932	.000	-29.0837	-12.8213
		4	-15.73750*	3.64932	.002	-23.8687	-7.6063
		5	-30.14750*	3.64932	.000	-38.2787	-22.0163
		6	-25.64250*	3.64932	.000	-33.7737	-17.5113
		7	-27.94250*	3.64932	.000	-36.0737	-19.8113

	8	-21.04250*	3.64932	.000	-29.1737	-12.9113
2	1	65.87250*	3.64932	.000	57.7413	74.0037
	3	44.92000*	4.21388	.000	35.5309	54.3091
	4	50.13500*	4.21388	.000	40.7459	59.5241
	5	35.72500*	4.21388	.000	26.3359	45.1141
	6	40.23000*	4.21388	.000	30.8409	49.6191
	7	37.93000*	4.21388	.000	28.5409	47.3191
	8	44.83000*	4.21388	.000	35.4409	54.2191
3	1	20.95250*	3.64932	.000	12.8213	29.0837
	2	-44.92000*	4.21388	.000	-54.3091	-35.5309
	4	5.21500	4.21388	.244	-4.1741	14.6041
	5	-9.19500	4.21388	.054	-18.5841	.1941
	6	-4.69000	4.21388	.292	-14.0791	4.6991
	7	-6.99000	4.21388	.128	-16.3791	2.3991
	8	-.09000	4.21388	.983	-9.4791	9.2991

4	1	15.73750*	3.64932	.002	7.6063	23.8687
	2	-50.13500*	4.21388	.000	-59.5241	-40.7459
	3	-5.21500	4.21388	.244	-14.6041	4.1741
	5	-14.41000*	4.21388	.007	-23.7991	-5.0209
	6	-9.90500*	4.21388	.041	-19.2941	-.5159
	7	-12.20500*	4.21388	.016	-21.5941	-2.8159
	8	-5.30500	4.21388	.237	-14.6941	4.0841
5	1	30.14750*	3.64932	.000	22.0163	38.2787
	2	-35.72500*	4.21388	.000	-45.1141	-26.3359
	3	9.19500	4.21388	.054	-.1941	18.5841
	4	14.41000*	4.21388	.007	5.0209	23.7991
	6	4.50500	4.21388	.310	-4.8841	13.8941
	7	2.20500	4.21388	.612	-7.1841	11.5941
	8	9.10500	4.21388	.056	-.2841	18.4941
6	1	25.64250*	3.64932	.000	17.5113	33.7737

	2	-40.23000*	4.21388	.000	-49.6191	-30.8409
	3	4.69000	4.21388	.292	-4.6991	14.0791
	4	9.90500*	4.21388	.041	.5159	19.2941
	5	-4.50500	4.21388	.310	-13.8941	4.8841
	7	-2.30000	4.21388	.597	-11.6891	7.0891
	8	4.60000	4.21388	.301	-4.7891	13.9891
7	1	27.94250*	3.64932	.000	19.8113	36.0737
	2	-37.93000*	4.21388	.000	-47.3191	-28.5409
	3	6.99000	4.21388	.128	-2.3991	16.3791
	4	12.20500*	4.21388	.016	2.8159	21.5941
	5	-2.20500	4.21388	.612	-11.5941	7.1841
	6	2.30000	4.21388	.597	-7.0891	11.6891
	8	6.90000	4.21388	.133	-2.4891	16.2891
8	1	21.04250*	3.64932	.000	12.9113	29.1737
	2	-44.83000*	4.21388	.000	-54.2191	-35.4409

	3	.09000	4.21388	.983	-9.2991	9.4791
	4	5.30500	4.21388	.237	-4.0841	14.6941
	5	-9.10500	4.21388	.056	-18.4941	.2841
	6	-4.60000	4.21388	.301	-13.9891	4.7891
	7	-6.90000	4.21388	.133	-16.2891	2.4891

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

TG LIVER

Descriptives

VAR00002

					95% Confidence Interval for Mean			
	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
1	3	21.4867	2.04998	1.18356	16.3942	26.5791	19.12	22.71
2	2	42.0100	.56569	.40000	36.9275	47.0925	41.61	42.41

3	2	32.2450	2.08597	1.47500	13.5033	50.9867	30.77	33.72
4	2	29.0000	.59397	.42000	23.6634	34.3366	28.58	29.42
5	2	29.7850	2.73650	1.93500	5.1985	54.3715	27.85	31.72
6	2	27.6850	1.42128	1.00500	14.9153	40.4547	26.68	28.69
7	2	24.9050	1.98697	1.40500	7.0528	42.7572	23.50	26.31
8	2	28.5300	2.02233	1.43000	10.3601	46.6999	27.10	29.96
Total	17	28.9871	6.11644	1.48346	25.8423	32.1318	19.12	42.41

Multiple Comparisons

Dependent Variable: VAR00002

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1	2	-20.52333*	1.69354	.000	-24.3544	-16.6923
		3	-10.75833*	1.69354	.000	-14.5894	-6.9273

	4	-7.51333*	1.69354	.002	-11.3444	-3.6823
	5	-8.29833*	1.69354	.001	-12.1294	-4.4673
	6	-6.19833*	1.69354	.005	-10.0294	-2.3673
	7	-3.41833	1.69354	.074	-7.2494	.4127
	8	-7.04333*	1.69354	.002	-10.8744	-3.2123
2	1	20.52333*	1.69354	.000	16.6923	24.3544
	3	9.76500*	1.85518	.001	5.5683	13.9617
	4	13.01000*	1.85518	.000	8.8133	17.2067
	5	12.22500*	1.85518	.000	8.0283	16.4217
	6	14.32500*	1.85518	.000	10.1283	18.5217
	7	17.10500*	1.85518	.000	12.9083	21.3017
	8	13.48000*	1.85518	.000	9.2833	17.6767
3	1	10.75833*	1.69354	.000	6.9273	14.5894
	2	-9.76500*	1.85518	.001	-13.9617	-5.5683
	4	3.24500	1.85518	.114	-.9517	7.4417

	5	2.46000	1.85518	.218	-1.7367	6.6567
	6	4.56000*	1.85518	.036	.3633	8.7567
	7	7.34000*	1.85518	.003	3.1433	11.5367
	8	3.71500	1.85518	.076	-.4817	7.9117
4	1	7.51333*	1.69354	.002	3.6823	11.3444
	2	-13.01000*	1.85518	.000	-17.2067	-8.8133
	3	-3.24500	1.85518	.114	-7.4417	.9517
	5	-.78500	1.85518	.682	-4.9817	3.4117
	6	1.31500	1.85518	.496	-2.8817	5.5117
	7	4.09500	1.85518	.055	-.1017	8.2917
	8	.47000	1.85518	.806	-3.7267	4.6667
5	1	8.29833*	1.69354	.001	4.4673	12.1294
	2	-12.22500*	1.85518	.000	-16.4217	-8.0283
	3	-2.46000	1.85518	.218	-6.6567	1.7367
	4	.78500	1.85518	.682	-3.4117	4.9817

	6	2.10000	1.85518	.287	-2.0967	6.2967
	7	4.88000*	1.85518	.027	.6833	9.0767
	8	1.25500	1.85518	.516	-2.9417	5.4517
6	1	6.19833*	1.69354	.005	2.3673	10.0294
	2	-14.32500*	1.85518	.000	-18.5217	-10.1283
	3	-4.56000*	1.85518	.036	-8.7567	-.3633
	4	-1.31500	1.85518	.496	-5.5117	2.8817
	5	-2.10000	1.85518	.287	-6.2967	2.0967
	7	2.78000	1.85518	.168	-1.4167	6.9767
	8	-.84500	1.85518	.660	-5.0417	3.3517
7	1	3.41833	1.69354	.074	-.4127	7.2494
	2	-17.10500*	1.85518	.000	-21.3017	-12.9083
	3	-7.34000*	1.85518	.003	-11.5367	-3.1433
	4	-4.09500	1.85518	.055	-8.2917	.1017
	5	-4.88000*	1.85518	.027	-9.0767	-.6833

	6	-2.78000	1.85518	.168	-6.9767	1.4167
	8	-3.62500	1.85518	.082	-7.8217	.5717
8	1	7.04333*	1.69354	.002	3.2123	10.8744
	2	-13.48000*	1.85518	.000	-17.6767	-9.2833
	3	-3.71500	1.85518	.076	-7.9117	.4817
	4	-.47000	1.85518	.806	-4.6667	3.7267
	5	-1.25500	1.85518	.516	-5.4517	2.9417
	6	.84500	1.85518	.660	-3.3517	5.0417
	7	3.62500	1.85518	.082	-.5717	7.8217

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

TG PLASMA

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	2	42.5200	2.84257	2.01000	16.9805	68.0595	40.51	44.53
2	2	93.6100	6.44881	4.56000	35.6697	151.5503	89.05	98.17
3	2	48.5350	2.58094	1.82500	25.3462	71.7238	46.71	50.36
4	2	49.0850	1.29401	.91500	37.4588	60.7112	48.17	50.00
5	2	62.4050	1.54856	1.09500	48.4917	76.3183	61.31	63.50
6	2	45.9850	25.29321	17.88500	-181.2655	273.2355	28.10	63.87
7	2	24.4500	6.19426	4.38000	-31.2032	80.1032	20.07	28.83
8	2	25.0000	1.28693	.91000	13.4374	36.5626	24.09	25.91
Total	16	48.9488	22.41969	5.60492	37.0021	60.8954	20.07	98.17

Multiple Comparisons

Dependent Variable: VAR00002

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1	2	-51.09000*	9.61881	.001	-73.2710	-28.9090
		3	-6.01500	9.61881	.549	-28.1960	16.1660
		4	-6.56500	9.61881	.514	-28.7460	15.6160
		5	-19.88500	9.61881	.073	-42.0660	2.2960
		6	-3.46500	9.61881	.728	-25.6460	18.7160
		7	18.07000	9.61881	.097	-4.1110	40.2510
		8	17.52000	9.61881	.106	-4.6610	39.7010
	2	1	51.09000*	9.61881	.001	28.9090	73.2710
		3	45.07500*	9.61881	.002	22.8940	67.2560
		4	44.52500*	9.61881	.002	22.3440	66.7060
		5	31.20500*	9.61881	.012	9.0240	53.3860
		6	47.62500*	9.61881	.001	25.4440	69.8060

	7	69.16000*	9.61881	.000	46.9790	91.3410
	8	68.61000*	9.61881	.000	46.4290	90.7910
3	1	6.01500	9.61881	.549	-16.1660	28.1960
	2	-45.07500*	9.61881	.002	-67.2560	-22.8940
	4	-.55000	9.61881	.956	-22.7310	21.6310
	5	-13.87000	9.61881	.187	-36.0510	8.3110
	6	2.55000	9.61881	.798	-19.6310	24.7310
	7	24.08500*	9.61881	.037	1.9040	46.2660
	8	23.53500*	9.61881	.040	1.3540	45.7160
4	1	6.56500	9.61881	.514	-15.6160	28.7460
	2	-44.52500*	9.61881	.002	-66.7060	-22.3440
	3	.55000	9.61881	.956	-21.6310	22.7310
	5	-13.32000	9.61881	.204	-35.5010	8.8610
	6	3.10000	9.61881	.755	-19.0810	25.2810
	7	24.63500*	9.61881	.034	2.4540	46.8160

	8	24.08500*	9.61881	.037	1.9040	46.2660
5	1	19.88500	9.61881	.073	-2.2960	42.0660
	2	-31.20500*	9.61881	.012	-53.3860	-9.0240
	3	13.87000	9.61881	.187	-8.3110	36.0510
	4	13.32000	9.61881	.204	-8.8610	35.5010
	6	16.42000	9.61881	.126	-5.7610	38.6010
	7	37.95500*	9.61881	.004	15.7740	60.1360
	8	37.40500*	9.61881	.005	15.2240	59.5860
6	1	3.46500	9.61881	.728	-18.7160	25.6460
	2	-47.62500*	9.61881	.001	-69.8060	-25.4440
	3	-2.55000	9.61881	.798	-24.7310	19.6310
	4	-3.10000	9.61881	.755	-25.2810	19.0810
	5	-16.42000	9.61881	.126	-38.6010	5.7610
	7	21.53500	9.61881	.056	-.6460	43.7160
	8	20.98500	9.61881	.061	-1.1960	43.1660

7	1	-18.07000	9.61881	.097	-40.2510	4.1110
	2	-69.16000*	9.61881	.000	-91.3410	-46.9790
	3	-24.08500*	9.61881	.037	-46.2660	-1.9040
	4	-24.63500*	9.61881	.034	-46.8160	-2.4540
	5	-37.95500*	9.61881	.004	-60.1360	-15.7740
	6	-21.53500	9.61881	.056	-43.7160	.6460
	8	-.55000	9.61881	.956	-22.7310	21.6310
8	1	-17.52000	9.61881	.106	-39.7010	4.6610
	2	-68.61000*	9.61881	.000	-90.7910	-46.4290
	3	-23.53500*	9.61881	.040	-45.7160	-1.3540
	4	-24.08500*	9.61881	.037	-46.2660	-1.9040
	5	-37.40500*	9.61881	.005	-59.5860	-15.2240
	6	-20.98500	9.61881	.061	-43.1660	1.1960
	7	.55000	9.61881	.956	-21.6310	22.7310

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

HDL-C

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	2	54.3800	2.37588	1.68000	33.0336	75.7264	52.70	56.06
2	2	16.0050	1.37886	.97500	3.6165	28.3935	15.03	16.98
3	2	47.9250	1.25158	.88500	36.6800	59.1700	47.04	48.81
4	2	51.1050	3.50018	2.47500	19.6571	82.5529	48.63	53.58
5	2	55.7000	2.99813	2.12000	28.7628	82.6372	53.58	57.82
6	2	49.5100	8.00445	5.66000	-22.4071	121.4271	43.85	55.17
7	2	53.3150	1.12430	.79500	43.2136	63.4164	52.52	54.11
8	2	48.6300	5.50129	3.89000	-.7971	98.0571	44.74	52.52
Total	16	47.0712	12.75577	3.18894	40.2742	53.8683	15.03	57.82

Multiple Comparisons

Dependent Variable: VAR00002

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1	2	38.37500*	3.96787	.000	29.2251	47.5249
		3	6.45500	3.96787	.142	-2.6949	15.6049
		4	3.27500	3.96787	.433	-5.8749	12.4249
		5	-1.32000	3.96787	.748	-10.4699	7.8299
		6	4.87000	3.96787	.255	-4.2799	14.0199
		7	1.06500	3.96787	.795	-8.0849	10.2149
		8	5.75000	3.96787	.185	-3.3999	14.8999
	2	1	-38.37500*	3.96787	.000	-47.5249	-29.2251
		3	-31.92000*	3.96787	.000	-41.0699	-22.7701
		4	-35.10000*	3.96787	.000	-44.2499	-25.9501

	5	-39.69500*	3.96787	.000	-48.8449	-30.5451
	6	-33.50500*	3.96787	.000	-42.6549	-24.3551
	7	-37.31000*	3.96787	.000	-46.4599	-28.1601
	8	-32.62500*	3.96787	.000	-41.7749	-23.4751
3	1	-6.45500	3.96787	.142	-15.6049	2.6949
	2	31.92000*	3.96787	.000	22.7701	41.0699
	4	-3.18000	3.96787	.446	-12.3299	5.9699
	5	-7.77500	3.96787	.086	-16.9249	1.3749
	6	-1.58500	3.96787	.700	-10.7349	7.5649
	7	-5.39000	3.96787	.211	-14.5399	3.7599
	8	-.70500	3.96787	.863	-9.8549	8.4449
4	1	-3.27500	3.96787	.433	-12.4249	5.8749
	2	35.10000*	3.96787	.000	25.9501	44.2499
	3	3.18000	3.96787	.446	-5.9699	12.3299
	5	-4.59500	3.96787	.280	-13.7449	4.5549

	6	1.59500	3.96787	.698	-7.5549	10.7449
	7	-2.21000	3.96787	.593	-11.3599	6.9399
	8	2.47500	3.96787	.550	-6.6749	11.6249
5	1	1.32000	3.96787	.748	-7.8299	10.4699
	2	39.69500*	3.96787	.000	30.5451	48.8449
	3	7.77500	3.96787	.086	-1.3749	16.9249
	4	4.59500	3.96787	.280	-4.5549	13.7449
	6	6.19000	3.96787	.157	-2.9599	15.3399
	7	2.38500	3.96787	.564	-6.7649	11.5349
	8	7.07000	3.96787	.113	-2.0799	16.2199
6	1	-4.87000	3.96787	.255	-14.0199	4.2799
	2	33.50500*	3.96787	.000	24.3551	42.6549
	3	1.58500	3.96787	.700	-7.5649	10.7349
	4	-1.59500	3.96787	.698	-10.7449	7.5549
	5	-6.19000	3.96787	.157	-15.3399	2.9599

	7	-3.80500	3.96787	.366	-12.9549	5.3449
	8	.88000	3.96787	.830	-8.2699	10.0299
7	1	-1.06500	3.96787	.795	-10.2149	8.0849
	2	37.31000*	3.96787	.000	28.1601	46.4599
	3	5.39000	3.96787	.211	-3.7599	14.5399
	4	2.21000	3.96787	.593	-6.9399	11.3599
	5	-2.38500	3.96787	.564	-11.5349	6.7649
	6	3.80500	3.96787	.366	-5.3449	12.9549
	8	4.68500	3.96787	.272	-4.4649	13.8349
8	1	-5.75000	3.96787	.185	-14.8999	3.3999
	2	32.62500*	3.96787	.000	23.4751	41.7749
	3	.70500	3.96787	.863	-8.4449	9.8549
	4	-2.47500	3.96787	.550	-11.6249	6.6749
	5	-7.07000	3.96787	.113	-16.2199	2.0799
	6	-.88000	3.96787	.830	-10.0299	8.2699

	7	-4.68500	3.96787	.272	-13.8349	4.4649

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

TP

Multiple Comparisons

Dependent Variable:VAR00002

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1	2	-6.90500*	.63365	.000	-8.3662	-5.4438
		3	-1.24500	.63365	.085	-2.7062	.2162
		4	-.90500	.63365	.191	-2.3662	.5562
		5	-1.44500	.63365	.052	-2.9062	.0162
		6	-2.56500*	.63365	.004	-4.0262	-1.1038
		7	-2.05500*	.63365	.012	-3.5162	-.5938
		8	-1.87000*	.63365	.018	-3.3312	-.4088

2	1	6.90500*	.63365	.000	5.4438	8.3662
	3	5.66000*	.63365	.000	4.1988	7.1212
	4	6.00000*	.63365	.000	4.5388	7.4612
	5	5.46000*	.63365	.000	3.9988	6.9212
	6	4.34000*	.63365	.000	2.8788	5.8012
	7	4.85000*	.63365	.000	3.3888	6.3112
	8	5.03500*	.63365	.000	3.5738	6.4962
3	1	1.24500	.63365	.085	-.2162	2.7062
	2	-5.66000*	.63365	.000	-7.1212	-4.1988
	4	.34000	.63365	.606	-1.1212	1.8012
	5	-.20000	.63365	.760	-1.6612	1.2612
	6	-1.32000	.63365	.071	-2.7812	.1412
	7	-.81000	.63365	.237	-2.2712	.6512
	8	-.62500	.63365	.353	-2.0862	.8362
4	1	.90500	.63365	.191	-.5562	2.3662

	2	-6.00000*	.63365	.000	-7.4612	-4.5388
	3	-.34000	.63365	.606	-1.8012	1.1212
	5	-.54000	.63365	.419	-2.0012	.9212
	6	-1.66000*	.63365	.031	-3.1212	-.1988
	7	-1.15000	.63365	.107	-2.6112	.3112
	8	-.96500	.63365	.166	-2.4262	.4962
5	1	1.44500	.63365	.052	-.0162	2.9062
	2	-5.46000*	.63365	.000	-6.9212	-3.9988
	3	.20000	.63365	.760	-1.2612	1.6612
	4	.54000	.63365	.419	-.9212	2.0012
	6	-1.12000	.63365	.115	-2.5812	.3412
	7	-.61000	.63365	.364	-2.0712	.8512
	8	-.42500	.63365	.521	-1.8862	1.0362
6	1	2.56500*	.63365	.004	1.1038	4.0262
	2	-4.34000*	.63365	.000	-5.8012	-2.8788

	3	1.32000	.63365	.071	-.1412	2.7812
	4	1.66000*	.63365	.031	.1988	3.1212
	5	1.12000	.63365	.115	-.3412	2.5812
	7	.51000	.63365	.444	-.9512	1.9712
	8	.69500	.63365	.305	-.7662	2.1562
7	1	2.05500*	.63365	.012	.5938	3.5162
	2	-4.85000*	.63365	.000	-6.3112	-3.3888
	3	.81000	.63365	.237	-.6512	2.2712
	4	1.15000	.63365	.107	-.3112	2.6112
	5	.61000	.63365	.364	-.8512	2.0712
	6	-.51000	.63365	.444	-1.9712	.9512
	8	.18500	.63365	.778	-1.2762	1.6462
8	1	1.87000*	.63365	.018	.4088	3.3312
	2	-5.03500*	.63365	.000	-6.4962	-3.5738
	3	.62500	.63365	.353	-.8362	2.0862

	4	.96500	.63365	.166	-.4962	2.4262
	5	.42500	.63365	.521	-1.0362	1.8862
	6	-.69500	.63365	.305	-2.1562	.7662
	7	-.18500	.63365	.778	-1.6462	1.2762

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

NO

Descriptives

VAR00002

					95% Confidence Interval for Mean			
	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
1	2	36.9500	17.74838	12.55000	-122.5129	196.4129	24.40	49.50
2	2	59.9500	2.89914	2.05000	33.9023	85.9977	57.90	62.00
3	2	30.1000	7.63675	5.40000	-38.5135	98.7135	24.70	35.50

4	2	41.8000	.84853	.60000	34.1763	49.4237	41.20	42.40
5	2	41.5000	1.13137	.80000	31.3350	51.6650	40.70	42.30
6	2	40.8500	2.61630	1.85000	17.3435	64.3565	39.00	42.70
7	2	40.5500	25.80940	18.25000	-191.3382	272.4382	22.30	58.80
8	2	40.2500	4.73762	3.35000	-2.3158	82.8158	36.90	43.60
Total	16	41.4938	11.73948	2.93487	35.2382	47.7493	22.30	62.00

Multiple Comparisons

Dependent Variable:VAR00002

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1	2	-23.00000	11.61435	.083	-49.7827	3.7827
		3	6.85000	11.61435	.572	-19.9327	33.6327
		4	-4.85000	11.61435	.687	-31.6327	21.9327

	5	-4.55000	11.61435	.705	-31.3327	22.2327
	6	-3.90000	11.61435	.746	-30.6827	22.8827
	7	-3.60000	11.61435	.765	-30.3827	23.1827
	8	-3.30000	11.61435	.784	-30.0827	23.4827
2	1	23.00000	11.61435	.083	-3.7827	49.7827
	3	29.85000*	11.61435	.033	3.0673	56.6327
	4	18.15000	11.61435	.157	-8.6327	44.9327
	5	18.45000	11.61435	.151	-8.3327	45.2327
	6	19.10000	11.61435	.139	-7.6827	45.8827
	7	19.40000	11.61435	.133	-7.3827	46.1827
	8	19.70000	11.61435	.128	-7.0827	46.4827
3	1	-6.85000	11.61435	.572	-33.6327	19.9327
	2	-29.85000*	11.61435	.033	-56.6327	-3.0673
	4	-11.70000	11.61435	.343	-38.4827	15.0827
	5	-11.40000	11.61435	.355	-38.1827	15.3827

	6	-10.75000	11.61435	.382	-37.5327	16.0327
	7	-10.45000	11.61435	.395	-37.2327	16.3327
	8	-10.15000	11.61435	.408	-36.9327	16.6327
4	1	4.85000	11.61435	.687	-21.9327	31.6327
	2	-18.15000	11.61435	.157	-44.9327	8.6327
	3	11.70000	11.61435	.343	-15.0827	38.4827
	5	.30000	11.61435	.980	-26.4827	27.0827
	6	.95000	11.61435	.937	-25.8327	27.7327
	7	1.25000	11.61435	.917	-25.5327	28.0327
	8	1.55000	11.61435	.897	-25.2327	28.3327
5	1	4.55000	11.61435	.705	-22.2327	31.3327
	2	-18.45000	11.61435	.151	-45.2327	8.3327
	3	11.40000	11.61435	.355	-15.3827	38.1827
	4	-.30000	11.61435	.980	-27.0827	26.4827
	6	.65000	11.61435	.957	-26.1327	27.4327

	7	.95000	11.61435	.937	-25.8327	27.7327
	8	1.25000	11.61435	.917	-25.5327	28.0327
6	1	3.90000	11.61435	.746	-22.8827	30.6827
	2	-19.10000	11.61435	.139	-45.8827	7.6827
	3	10.75000	11.61435	.382	-16.0327	37.5327
	4	-.95000	11.61435	.937	-27.7327	25.8327
	5	-.65000	11.61435	.957	-27.4327	26.1327
	7	.30000	11.61435	.980	-26.4827	27.0827
	8	.60000	11.61435	.960	-26.1827	27.3827
7	1	3.60000	11.61435	.765	-23.1827	30.3827
	2	-19.40000	11.61435	.133	-46.1827	7.3827
	3	10.45000	11.61435	.395	-16.3327	37.2327
	4	-1.25000	11.61435	.917	-28.0327	25.5327
	5	-.95000	11.61435	.937	-27.7327	25.8327
	6	-.30000	11.61435	.980	-27.0827	26.4827

		8	.30000	11.61435	.980	-26.4827	27.0827
	8	1	3.30000	11.61435	.784	-23.4827	30.0827
		2	-19.70000	11.61435	.128	-46.4827	7.0827
		3	10.15000	11.61435	.408	-16.6327	36.9327
		4	-1.55000	11.61435	.897	-28.3327	25.2327
		5	-1.25000	11.61435	.917	-28.0327	25.5327
		6	-.60000	11.61435	.960	-27.3827	26.1827
		7	-.30000	11.61435	.980	-27.0827	26.4827

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

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	Minimum	Maximum

					Lower Bound	Upper Bound		
1	2	1.5000	.24042	.17000	-.6601	3.6601	1.33	1.67
2	2	8.4050	.17678	.12500	6.8167	9.9933	8.28	8.53
3	2	2.7450	.12021	.08500	1.6650	3.8250	2.66	2.83
4	2	2.4050	.60104	.42500	-2.9951	7.8051	1.98	2.83
5	2	2.9450	.48790	.34500	-1.4386	7.3286	2.60	3.29
6	2	4.0650	.04950	.03500	3.6203	4.5097	4.03	4.10
7	2	3.5550	1.56271	1.10500	-10.4854	17.5954	2.45	4.66
8	2	3.3700	.25456	.18000	1.0829	5.6571	3.19	3.55
Total	16	3.6238	2.06426	.51607	2.5238	4.7237	1.33	8.53