EFFECT OF ETHANOL EXTRACT OF Ocimum gratissimum ON CARDIOVASCULAR DISEASE RISK FACTOR OF CYANIDE-INDUCED RABBITS

BY

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CERTIFICATION

We the designated certify that this project work was carried out and presented by NWANKWOR FRANCIS IFEANYI in partial fulfillment of the requirements for the award of Bachelor of Science (**B.Sc**, Hons) degree in Biochemistry, in the Department of Biochemistry.

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DEDICATION

This work is dedicated to my beloved parents Mr. Nwankwor Raphael and Mrs. Nwankwor Anastacia for their unconditional love and support in my life and to Almighty God.

ACKNOWLEDGMENTS

First and Foremost, heartfelt appreciation to God Almighty for unending grace to complete this exercise successfully.

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God bless you all.

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ABSTRACT

Cyanide poisoning poses a significant threat to cardiovascular health, leading to the development of cardiovascular disease (CVD) risk factors such as dyslipidemia, oxidative stress, and endothelial dysfunction. Ocimum gratissimum (OG), commonly known as scent leaf, is a medicinal plant with potential cardioprotective properties. This study aimed to investigate the effect of the ethanol extract of OG on CVD risk factors in cyanide-induced rabbits. Fifteen rabbits were randomly divided into five groups: group one(drug), group two(ethanol extract), group three(ethanol extract), group four(cyanide), group five(control). Cyanide-induced rabbits were orally administered OG extract and drug(Sylimarin) for 21 days. Blood samples and organs(heart) were collected for biochemical analysis of CVD risk factors, including total protein profile, cholesterol levels, arterogenic coefficient, cardiac risk ratio, vitamin, and nitric oxides concentration. Certain experimental groups displayed promising changes, such as elevated HDL-C levels, improved endothelial function, and enhanced cardiac muscle function, others exhibited adverse alterations in lipid profile and endothelial function. These findings underscore the complex nature of herbal remedies and emphasize the importance of empirical validation to ensure their safety and efficacy in treating cardiovascular diseases.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

The fragrant herb Ocimum gratissimum has spread widely throughout tropical and subtropical areas of the globe. Since it has escaped cultivation, it has spread to disturbed natural vegetation, savannas, coastal thickets, and riparian areas, where it grows as a weed in waste areas, pastures, and roadsides. This species has many tiny, easily dispersed seeds that can be carried by wind, animals, people, and soil and garden waste. Ocimum gratissimum has the capacity to spread once it is established, creating dense, monospecific thickets that displace native vegetation and lower native biodiversity.

Ocimum gratissimum is a fragrant, evergreen shrub that grows throughout tropical regions, including Brazil, India, Vietnam, Rwanda, and Nigeria. Its antibacterial qualities were assessed in relation to the ethanolic extract of Ocimium gratisimum leaves, which are used in traditional medicine to treat a variety of conditions, including respiratory tract, skin, and gastrointestinal infections. The fresh leaves had a moisture content of 81.35%, a protein content of 1.21% and an ash content of 0.57%. On a fresh weight basis mineral content was as follows: phosphorus 52.4, selenium 0.007, iron 7.9 and zinc 1.5 µg g-1. Many bioactive substances are present in it, such as oxalic acid, eugenol, thymol, rosmarinic acid, glycosides, alkaloids, phenols, terpenoids, flavonoids, saponins, steroids, and tannins.

Ocimum oil and its constituents and derivatives are used as flavoring agents in the food, pharmaceutical, herbal, perfumery, and flavoring industries worldwide, demonstrating the oil's economic significance. In addition to being less expensive, Ocimum plants' safety, effectiveness, and global availability are significant benefits in a variety of treatments.

1.1.1 AIM AND OBJECTIVE OF THE STUDY

This study aimed to investigate the potential cardiovascular protective effects of the ethanol extract of Ocimum gratissimum (OG) on cyanide-induced rabbits, focusing on cardiovascular disease risk factors.

1.1. 2 OBJECTIVES

1. To poison normal rabbit with potassium cyanide
2. To determine the effect of cyanide poison on the rabbit.
3. Measure the level of parameters on cardiac matters.

1.2 LITERATURE REVIEW

1.2.1 Scientific Classsification of Plant Species

Domain……Eukaryota

Kingdom……. Plantae

Subkingdom……....Tracheobionta

Phylum…….....Spermatophyta

Subphylum..... Angiospermae

Class……Dicotyledonae

Order…….. Lamiales

Family…… Lamiaceae

Genus……. Ocimum

Specie…….Ocimum gratissimum

1.2.2 Nomenclature

Scientific Name: Ocimum gratissimum Lamiaceae

Common Name(s): scent leaf, basil, clove basil, alfavaca.

Nigerian Name(s): Nchanwu (Igbo), Efinrin(Yoruba), Daidoya (Hausa).

1.2.3 Description

The aromatic herbaceous plant Ocimum gratissimum is also referred to as alfavaca, basil, or basil-clove. It is a member of the Lamiaceae family (species gratissimum, genus Ocimum) (Nweze and Eze, 2009). Its stem is erect, branched, round-quadrangular, and woody at the base. Its opposite, slender, and marginalized leaves are about 1-3 centimeters tall.

There are roughly 7200 species of herbs, shrubs, trees, and vines in the Lamiaceae family, which is distributed over 236 genera (Stevens, 2017). About 65 species make up this group, the majority of which are indigenous to Africa (Flora Mesoamericana, 2018). For thousands of years, many Ocimum species have been grown, especially in the Mediterranean Basin and southern Asian regions (Basin, 2012). Numerous forms of Ocimum gratissimum exist; it is a highly dependable polymorphic species, many of which were once considered distinct species and subspecies.

1.2.4 Habitat

Native to Africa, Madagascar, southern Asia, and the Bismarck Archipelago, it has naturalized in Brazil, Bolivia, Polynesia, Hawaii, Mexico, Panama, the West Indies, and Brazil.

1.2.5 Ecological Conditions

The cryptogenic species Ocimum gratissimum has two common forms. Particular habitats for the typical form include secondarized forests and degraded dry thickets, as well as crops, villages, and coastal cliffs that extend up to the heights. The form with broad leaves is more hygrophilic. It grows in villages and degraded forests in mesophilic and hygrophilic regions. This species can grow to a medium height and form hedges alongside roads in semi-shaded

areas. With considerable variability, it grows throughout the tropics and subtropics, but primarily in tropical Africa and India (Rhoda and Negimote, 2015).

1.2.6 Propagation

Cuttings or seeds can be used to propagate Ocimum gratissimum. The seeds take a couple of days to germinate. Full sun is ideal for Ocimum gratissimum germination and growth. It can reach a height of five feet. Start the seeds indoors approximately six weeks prior to the last date of frost if you are cultivating a garden in a colder climate. Because scent leaf seeds are small, it is best to plant them directly on top of moist potting soil without covering them. Scent Leaf Plants can be grown via stems. Simply chop off a mature branch and plant it in the ground. It will take 28 days for it to start growing.

Image 1: The leaves of Ocimum gratissimum

Source: Google images

Image 2: Matured stem and leaves of Ocimum gratissimum .

Source: Google images

1.2.7 Phytochemistry.

The phenolic compounds found in Ocimum gratissimum include rosmarinic acid, sinapic acid, salvigenin, gallic acid, catechins, methyl eugenol, caffeic acid, L-caftaric, ellagic acid, trans-ferulic acid, L-chicoric acid, and flavonoids such as xanthomicrol, cirsimaritin, rutin, apigenin, kaempferol, vicenin-2, luteolin 5-O-glucoside, luteolin 7-O-glucoside, 7,4,′-dimethyl ether, vitexin, isovitexin, nepetoidin A, quercetin 3-O-glucoside, nevadensin, cirsimaritin, hymenoxin, myricetin, basilimoside, morin, isothymusin (Grayer et al., 2000; Costa et al., 2012; Ouyang et al., 2013; Casanova et al., 2014; Venuprasad et al., 2014; Ajayi et al., 2019), epicatechin, quercitrin, quercetin (Irondi et al., 2016), and triterpenes (oleanolic, pomolic acid, ursolic acids, and tormentic acid) (Dzoyem et al., 2021).

1.2.8 Traditional Uses

Numerous ethnopharmacological surveys have reported that Ocimum gratissimum is a plant that is easily accessible to communities and is frequently used to treat a wide range of diseases (Ajayi et al., 2017a,b,c). Generally recognized to have medicinal properties, this odorous perennial plant can now be found on every continent. Its enormously broad medicinal potential in Africa differs from nation to nation (Kpoviessi et al., 2014). In Cameroon, the juice of its sheets is used to treat coughs, colds, giddiness, and headaches. Its infusions are considered pectoral and tonic. Several preparations of this plant are used in Côte d'Ivoire to treat ophthalmias, dermatoses, and ear infections (Kpoviessi et al., 2014). It is advised for treating diarrhea in Nigeria (Kpoviessi et al., 2014), and Sofowora (1970) suggested it for treating respiratory conditions and as an anthelminthic. In addition to treating pneumonia, it was also used to treat headaches, fevers, ophthalmic, and skin conditions. The plant's infusion is used as an antitussive to treat coughs in Togo. Purulent urethritis and haematuria are treated with its aqueous maceration, and the fresh juice from its leaves has

antidysentric and antidiarrheic qualities (Kpoviessi et al., 2014). In the Benin Republic, dystopias, pelvic aches, colic, candidoses, digestive dysmenorrhea, emesis, hemorrhoids (piles), and diarrhea are all treated with the aqueous maceration of its pulp or aerial portions. Hepatitis, cough, asthma, and wound infections are all treated with its stem decoction (Chah et al., 2006; Kpoviessi et al., 2014). Its leaf juice is used to treat malnourishment, fever, headaches, angina, and cephalalgias. In many different dishes, its inflorescences are used as aromatizers.

1.2.9 Anxiolytic activity

Anxiety is a mental illness that affects people of all ages, from young children to the elderly, and is characterized by unpleasant behavior and internal turmoil. Anxiety is frequently treated with benzodiazepines and other allopathic medications that non-selectively target gamma-aminobutyric acid (GABA) receptors (Rudolph and Knoflach, 2011). The latency of tonic and tonic-clonic seizures and death was found to be increased by 200 and 400 mg/kg of methanol or petroleum ether extract, according to Okoli et al. (2010). Additionally, they provided 50% defense against seizure-induced death in mice receiving treatment. Numerous investigations have demonstrated a connection between anti-anxiety properties and antioxidant activity (Hovatta et al., 2010). Glyoxalase-1 and glutathione-1 are the main proteins whose activities affect anxiety, although several other gene products also play a significant role in anxiety (Hovatta et al., 2005). O. gratissimum has been shown to have protective properties against lipid, protein, and DNA peroxidation (Venuprasad et al., 2014). After pre-treating SH-SY5Y human neuronal cells with the plant's leaf extract, lipid peroxidation inhibition was observed at an IC50 value of 735 g/mL, resulting in 44.8% protection against H2O2-induced DNA damage and 80% protection against AAPH-induced BSA oxidation. Results from the elevated plus maze and open field tests showed that the

plant, at a dosage of 400 mg/kg body weight, significantly reduced the anxiety of test mice (Venuprasad et al., 2014).

1.3 Antinociceptive activity

O. gratissimum is used in traditional medicine to alleviate painful conditions. The antinociceptive effects of O. gratissimum essential oil and two of its active ingredients, myrcene and eugenol, were studied (using the hot plate test and the formalin test) in classic pain models on neurogenic and inflammatory pain in murine pain models (Paula-Freire et al., 2013). During both the initial and subsequent phases of the formalin test, animals' pain was effectively decreased by O. gratissimum essential oil (40 mg/kg) and its active ingredients (10 mg/kg) eugenol and myrcene. These results corroborate the research conducted by other authors regarding the antinociceptive properties of O. gratissimum essential oil (Paula-Freire et al., 2013). In male Swiss mice, O. gratissimum essential oil at 30, 100, and 300 mg/kg was shown by Rabelo et al. (2003) to reduce inflammation and writhing. O. gratissimum has long been used in the treatment of pain; the findings of Tanko et al. (2008) indicate that it has a notable antinociceptive effect when administered at 1264.9 mg/kg body weight in rats using the hot plate technique and the acetic-acid-induced abdominal constriction test.

1.3.1 Neuroprotective activity

Brain function is enhanced by O. gratissimum leaf extract supplements. According to Ajayi et al. (2018), mice with behavioral impairment and depressive-like behavior were treated with O. gratissimum at body weights of 25, 50, and 100 mg/kg using the forced swim test (FST) and the open field test (OFT). As demonstrated during pre-treatment in Wistar rats with middle cerebral artery occlusion for 24 hours followed by 24 hours of reperfusion, Bora et al. (2011) found that the neuroprotective ability of O. gratissimum extract in cerebral ischaemia is mediated by its antioxidant properties.

1.3.2 Anti-anaemic activity

According to several research, O. gratissismum leaf extract can lessen toxicities that are imposed on Wistar rats' haematological indices. For instance, O. gratissimum leaf extract, at 400 mg/kg body weight, was shown by Akara et al. (2021) to decrease anemia in rats induced by phenylhydrazine. O. gratissimum extracts have been shown to have haematological effects on the body, as evidenced by increases in hemoglobin, packed cell volume, red blood cell counts, platelet counts, and neutrophil counts. Utilizing 500 mg/kg of the extract in addition to feed pellets resulted in a drop in the platelet count (Ofem et al., 2012). According to Akara et al. (2021), the haematopoietic traits discovered in their study could be caused by the iron and vitamins included in the O. gratissimum aqueous extract.

1.3.3 Wound healing properties

Chang et al. (2021) found that 100 μg/mL O. gratissimum protected against UVC-induced inhibition of skin cell migration and proliferation and restored cell activity, making it a powerful natural wound care agent. Orafidiya et al. (2006) report that S. aureus was affected by the antibacterial properties of a formulation that contained 2% O. gratissimum and honey as a surfactant. It showed that the antibacterial activity of ocimum oil is dependent on the net electrical charge on the surfactant used in its production. Together with honey's known ability to heal wounds, the 2% ocimum oil in honey formulation's outstanding antibacterial activity raises the possibility that it could be helpful as a topical antiseptic agent for wounds (Orafidiya et al., 2006).

1.4 POTASSIUM CYANIDE

Potassium cyanide is a highly toxic substance that is used in many industrial processes but is notoriously deadly. It is a compound with the KCN formula. It is an extremely soluble salt in water that has a colorless appearance akin to sugar. Organic synthesis, electroplating, and gold mining employ the majority of KCN. Smaller uses include chemical gilding and buffing of jewelry. Due to its extreme toxicity, almost all humans can be killed by 200–300 milligrams of potassium cyanide. Little amounts of hydrogen cyanide are released by the moist solid as a result of hydrolysis, or reaction with water. Potassium cyanide is frequently described as smelling like bitter almonds. Potassium cyanide is said to have an acrid, bitter taste and a burning sensation akin to lye.

Potassium cyanide is a cyanide salt with the same proportion of cyanide anions and potassium cations. It functions as a neurotoxin, an inhibitor of superoxide dismutase (EC 1.15.1.1), and an inhibitor of cytochrome c oxidase (EC 1.9.3.1). It is a potassium salt, cyanide salt, and one-carbon compound. An extremely toxic substance that inhibits numerous metabolic processes, but it has been demonstrated to have a particularly strong effect on heme proteins and enzymes.

1.4.1. STRUCTURE OF POTASSIUM CYANIDE

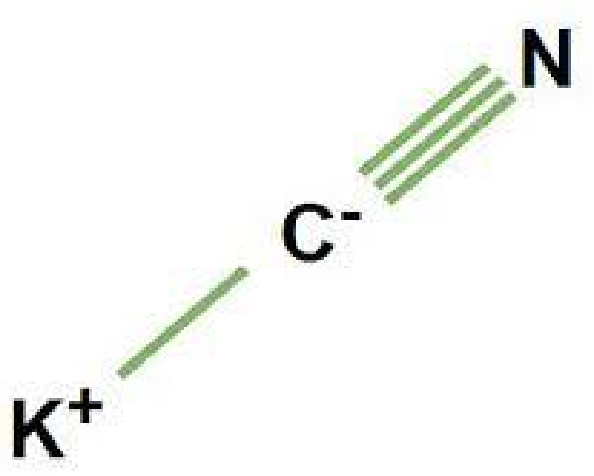

Plate 3: Structure of potassium cyanide
Source: Google images

1.4.2 PROPERTIES OF POTASSIUM CYANIDE

Potassium cyanide is a colorless, white, crystalline solid. It melts at 634.5°C and has a density of 1.52 g/ml. It is highly soluble in water. When heated, it breaks down quickly and gradually in the air. When hydrogen peroxide and potassium cyanide combine, a less dangerous cyanide derivative is created: KCN+ H2O2 -> KOCN+ H20. It has a bitter taste, burns when touched, and is poisonous.

1.4.3 PREPARATIONS OF POTASSIUM CYANIDE

Depending on its intended application, industries can prepare potassium cyanide in a variety of ways.
After a reaction between hydrogen cyanide and a potassium hydroxide aqueous solution:-
$HCN + KOH \rightarrow KCN + H_2O$

1.4.4 USES OF POTASSIUM CYANIDE

Potassium cyanide is widely used in the production of nitriles and carboxylic acids.
The breakdown of potassium ferrocyanide is accomplished with potassium cyanide.

In the colloidal process, KCN serves as a photographic fixer.
KCN is employed in the mining of metals, including gold.
Plastic and other organic synthesis are prepared using potassium cyanide.
Fumigation and electroplating.
Warehouses also make use of potassium cyanide.
KCN is used as insecticide by farmers.

1.4.5 EFFECT ON HEALTH

It is an extremely toxic substance that inhibits a variety of metabolic processes. It is poisonous when absorbed through open wounds on the skin. Toxic fumes are released during its breakdown when heated. Excessive exposure to KCN fumes can result in death and unconsciousness as well as headache, nausea, dizziness, anxiety, and racing heart.

1.5 CARDIOVASCULAR SYSTEM

The circulatory system is made up of your heart and blood vessels. The circulatory system's primary job is to supply your body's muscles, tissues, and organs with oxygen, nutrition, and hormones. The removal of waste from cells and organs for elimination by the body is another function of the circulatory system.

A system of veins and arteries (blood vessels) allows your heart to pump blood throughout your body. Your cardiovascular system is another name for your circulatory system. Vascular refers to blood vessels, and cardio refers to the heart.

1.5.1 OVERVIEW OF THE HEART

Situated in the middle of the mediastinum, the heart is a hollow, muscular organ that is conical in shape and enclosed by the pericardium. It is located posteriorly to the sternum's body, with two thirds on the left and one third on the right of the midline. The heart weighs about 255 g for females and 310 g for males, with measurements of 12 x 8.5 x 6 cm. It

supplies blood to different body parts so they can get the nutrients they need. The adjective cardi comes from the Greek word cardia, which is also the name of the heart.

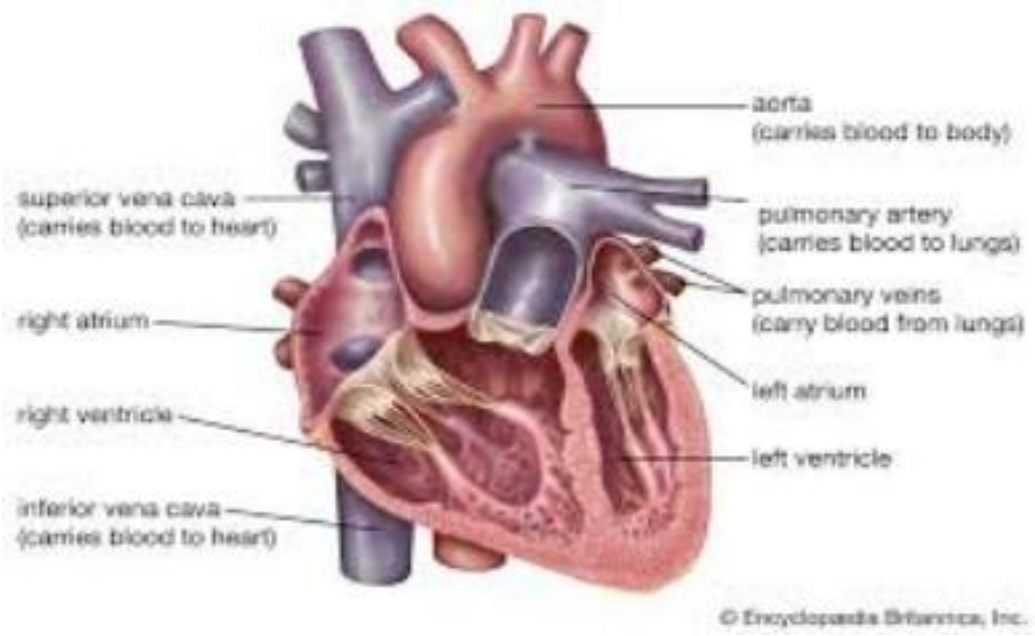

Image 3: The heart

Source: Google images

1.5.2 HEART VALVES

The heart's valves control blood flow in a single direction and stops it from regurgitating in the other. There are two sets of valves. Atrioventricular and semilunar valve pairs as well as other valves are found in two pairs in the heart. Four are included in it. Each of the heart's four valves has the same function, which is to permit blood to flow forward while obstructing backward flow. Every chamber has a heart valve protecting the exit.

An atrioventricular valve sits between the ventricles and the atria.

-tricuspid valve on the right side of the heart

-bicuspid valve on the left side of the heart

There are semilunar valves in the ventricles' outflow pathways.

The aortic valve on the left side of the heart

-pulmonary valve (heart's right side)

1.5.3 CARDIOVASCULAR DISEASE OVERVIEW

Cardiovascular disease (CVDs) encompass a range of conditions affecting the heart and blood vessels, such as rheumatic heart disease, coronary heart disease, and cerebrovascular disease. Over 40% of all CVD deaths are attributable to heart attacks and strokes, and among those under the age of 70, one-third happen prematurely (WHO, 2022).

1.5.4 CORONARY ARTERY DISEASE

Coronary artery disease, also known as coronary heart disease, is the most common type of heart disease. It takes place when plaque accumulates in the blood vessels that supply the heart. As a result, the plaque becomes harder and narrower, containing materials such as cholesterol. As a result, the heart receives less blood supply oxygen and nutrients. Over time, the heart muscle may weaken and lead to heart failure and arrhythmias. The accumulation of plaque in arteries is known as atherosclerosis. A heart attack may occur from plaque in arteries that bursts from blockages and prevents blood flow.

1.5.4 CONGENITAL HEART DEFECTS

A congenital cardiac defect results in a cardiac ailment from birth. Congenital cardiac abnormalities come in many different forms:

-Atypical heart valves: These heart valves may malfunction or allow blood to leak.

-Septal defects: a hole in the wall separating the heart's upper or lower chambers.

-Atherosia is the term for a missing heart valve.

Serious anatomical problems resulting from congenital heart disease include problems with abnormal connections between the main arteries supplying blood to the heart and the absence of a ventricle.

1.5.5 DILATED CARDIOMYOPATHY

The enlargement of the heart chambers due to dilated cardiomyopathy results in the contraction and thinned out heart muscle. Toxins, arrhythmias, and prior heart attacks are the most common causes of dilated cardiomyopathy, though genetics can occasionally play a role. A weaker, less efficient heart that has problems pumping blood is the result. Arrhythmia, heart failure, and blood clots in the heart are possible consequences.

1.5.6 HEART FAILURE

Heart failure is characterized by a person's heart continuing to beat, but not as well as it should. Problems with the pumping or relaxing function of the heart can lead to congestive heart failure. Heart failure can result from untreated coronary artery disease, high blood pressure, arrhythmias, and other conditions. The ability of the heart to pump or rest normally may be compromised by these conditions.

1.5.7 CARDIOVASCULAR DISEASE RISK FACTOR

Risk factors for cardiovascular disease are traits—both modifiable and non-modifiable—that raise the chance of getting the disease. In the general population, traditional CVD risk factors were found to confer an increased risk of CVD in the Framingham Heart Study. Traditional risk factors include smoking, diabetes, high blood pressure, male sex, older age, and family history (Mark et al., 2019).

Insulin resistance and the metabolic syndrome are additional risk factors. A number of CVD risk factors occur more frequently than predicted. The clustering of these traits, particularly the increased triglycerides, decreased HDL cholesterol, impaired fasting glucose, higher blood pressure, and greater abnormal adiposity, is thought to be explained by insulin resistance. The metabolic syndrome is defined as the presence of three or more of these five abnormalities; certain criteria are gender specific (Peter, 2013).

1.5.8 HOW DOES POTASSIUM CYANIDE AFFECT THE HEART

Potassium cyanide is a very harmful chemical that prevents cells from respiring by blocking the mitochondria's cytochrome c oxidase enzyme. As an organ with a high metabolic rate, the heart is especially vulnerable to disturbances in cellular respiration.

Potassium cyanide quickly attaches to cytochrome c oxidase upon ingestion or bloodstream entry, blocking the last stage of the electron transport chain in aerobic respiration. The severe impairment of cellular energy production caused by this inhibition causes tissue hypoxia (oxygen deprivation) to occur quickly.

Potassium cyanide can have significant effects on the heart, which primarily depends on aerobic metabolism to produce ATP for contraction.

CHAPTER TWO

MATERIALS AND METHODS

2.1 REAGENTS

The reagents used in this study were manufactured by Loba Chemie Pvt Ltd, India and they include:

- Pyrogallol
- Normal saline
- Distilled water, H2O
- Phosphate Buffered Hydrogen Peroxide
- Chloroform
- Ethanol
- Hydrochloric acid
- Picric acid
- Formalin
- TCA(Trichloroacetic acid)
- TBA (Thiobarbituric acid)
- Greiss reagent
- Ellaman's reagent
- Sodium chloride
- Potassium pomogamate
- Ascorbic acid
- Sulphuric acid

2.2 EQUIPMENT/ APPARATUS

The apparatus used in this study include:

- Electronic compact scale/ Weighing balance
- Centrifuge machine
- Water bath
- UV/Visible spectrophotometer
- Beakers
- Cuvette
- Syringes
- Needles
- Test tubes
- Test tubes rack
- Refrigerator
- Mortal and pestle
- Tag pins
- Cardboard papers
- Test tubes
- Measuring cylinder
- Micropipette
- pH meter
- Freezer
- Stirring rod
- Gloves

2.3 METHODOLOGY

2.3.1 PLANT COLLECTION AND IDENTIFICATION

Fresh leaves of Ocimum gratissimum were obtained in January, 2024 from a farmer in Benin city, Edo state.

2.3.2 PREPARATION OF ETHANOL EXTRACT OF Ocimum gratissimum

The freshly collected leaves of Ocimum gratissimum were separated by hands and shade dried properly for days to prevent degrading of its active constituents by rays of sunlight. The dried leaves and sticks was broken and reduced into smaller pieces and placed inside an empty bucket. 5l of absolute ethanol was added and the mixture was stirred intermittently for 72 hours. The resulting mixture was filtered with a sieve cloth. The filtrate was frizzed dried for days and the extract was kept in open air until the time of the experiment.

2.3.3 EXPERIMENTAL DESIGN

A total of young 15 New Zealand rabbits weight 275.9g-774.2g was used for this study. The animals were purchased from a local dealer and were kept in well ventilated wooden cage. They were maintained and acclimatized to diet and environment 28 days after arrival. In this experiment, the 15 rabbits were divided into 5 groups: group one(drug), group two(ethanol extract), group three(ethanol extract), group four(cyanide), group five(control). All group animals were fed with pellet grower and water in the morning and evening until the day of sacrifice.

2.3.4 MEASUREMENT OF BODY WEIGHTS

The weight of each rabbits were measured before and after acclimatization. This was done to ascertain the effect of the various feed constituents on their body weights. The table below shows the body weights before and after acclimatization:

Groups	Before Acclimatization (weight in grams)	After Acclimatization (weight in gram)
Group 1	531.0 607.5 533.2	810.2 675.2 788.7
Group 2	357.0 774.2 685.0	574.1 762.9 871.6
Group 3	541.6 541.8 644.0	693.4 739.5 (lost)
Cyanide group	562.0 712.9	562.0 712.9
Control group	685.0 485.3	514.9 275.6

2.3.5 INDUCTION OF POTASSIUM CYANIDE

All groups except group 5(control group) were induced with cyanide. The poison was induced by injecting freshly prepared potassium cyanide solution (7ml of cyanide and 20ml of distilled water). The poison was administered orally according to body weight using gavage.

2.3.6 ADMINISTRATION OF PLANT EXTRACT AND SILYMARIN DRUG

The rabbits in group 1 were orally administered with 70mg of silymarin drug while the rabbits in group 2 and 3 were orally administered 200mg/kg and 300mg/kg body weight respectively of the ethanol extract of Ocimum gratissimum for 21 days.

2.3.7 EXPERIMENTAL TERMINATION AND SACRIFICE

After the 21st day of treatment, the rabbits were allowed to fast overnight before being sacrificed the next morning. This involved anesthesia of each rabbit in a closed desiccator that contained drops of chloroform. After dissecting each rabbit, a 5-milliliter syringe was used to extract blood from the aorta into tubes holding lithium heparin and EDTA (tetraacetic acid) for hematological analysis. The plasma from the centrifuged blood sample in the lithium heparin was gathered and refrigerated for further examination. Following their excision, the heart, liver, kidney, and spleen were immediately rinsed in regular saline (0.9 NaCl) to remove blood stains. They were then dried between layers of Whatmann filter paper and weighed with a sensitive weighing balance. For the histopathology examination, 1g of each excised organ and 10 formalin were used.

2.3.8 BIOCHEMICAL ASSAY

TOTAL PROTEIN FOR HEART

Principle:

In an alkaline medium, proteins combine with the cupric ions in the Biuret reagent to generate a complex with a blue-violet hue. The quantity of proteins in the sample directly correlates with the color's intensity (Tietz 1995).

Assay Procedure:

Three labels were placed on the test tubes: "reagent blank," "standard," and "sample." Whereas the test tube holding the standard contained 0.1ml of Standard(Cal) and 0.1 ml of Solution R1, the reagent blank contained 0.1ml of distilled water and 1.0 ml of Solution R1. 0.1 ml of sample and 0.1ml of R1 were present in the sample tube. Each test tube's contents was incubated at 25°C for 30 minutes. At a wavelength of 546 nm, the absorbance of the sample, the standard, and the reagent blank were measured.

Calculations

Total protein concentration = Absorbance of sample x standard concentration

Absorbance of standard

DETERMINATION OF TRIGLYCERIDES

Principles:

Enzymatic determination of triglycerides is based on the following reactions:

$$TGL + H2O \xrightarrow{\text{Lipoprotein lipase}} \text{Glycerol + fatty acid}$$

$$\text{Glycerol-3-phosphate + ATP} \xrightarrow[Mg+]{\text{Glycerol kinase}} \text{Glycerol-3-phosphate + AD}$$

$$\text{Glycerol-3-phosphate + O2} \xrightarrow{\text{GPO}} \text{Dihydroxyacetone phosphate + H2O2}$$

$$2H2O2 + \text{Aminoantipyrine + TOPS} \xrightarrow{\text{POD}} \text{Violet Coloured complex}$$

Where:

GPO: Glycerol-3-phosphate oxidase

GK: Glycerol kinase

LPL: Lipoprotein Lipase

Assay Procedure

Three labels were placed on the test tubes: "sample," "standard," and "reagent blank." In the test tube holding the standard, there was 0.1ml of standard and 0.5ml of reagent, whereas the reagent blank contained 1.0ml of reagent. Sample and reagent volumes were 0.1 and 0.5ml, respectively, in the sample tube. After filling each test tube, it was incubated at 37°C for 10minutes. At a wavelength of 540nm, the absorbance of the sample, the standard, and the reagent blank were measured.

Calculation:

Triglycerides Con. (me/dL) = Absorbance of sample ×Absorbance of Standard X 200

DETERMINATION OF TOTAL CHOLESTEROL CONCENTRATION

Principle

First, cholesterol esterase hydrolyzes the cholesterol ester in the samples to produce free fatty acids and cholesterol. Hydrogen peroxide, cholestene-3-one, and cholesterol oxidase then oxidize the cholesterol. In the presence of phenol, 4 aminoantipyrene, and peroxidase, hydrogen peroxide is transformed into the indicator quinoneimine, which can be measured at 500nm using a spectrophotometer (Trinder, 1969).

Procedure

To the appropriate test tubes with the labels "standard," "blank," and "sample." 10µL of plasma cholesterol standard, and distilled water were added. The tubes were then filled with 1000µL of cholesterol reagent. After mixing the solution, it was incubated at 25°C for 10 minutes. At 500 nm, the absorbance was measured in less than 60 minutes against the reagent blank. Calculations were made to determine the cholesterol content.

Calculation

Concentration of cholesterol in test= (Asample x concentration of standard) / Astandard

DETERMINATION OF PLASMA HDL-CHOLESTEROL COMCENTRATION

Principle

Adding phosphotungstic acid in the presence of magnesium ions causes the low density lipoprotein (LDL and VLDL) and chylomicrons fractions to precipitate quantitatively. Centrifugation is followed by measurement of the cholesterol content in the high density lipoprotein (HDL) fraction that stays in the supernatant (Lopes-Virell, 1977).

Assay procedure

Test tubes labeled sample and standard were filled with precisely 200µL of triacylglycerol standard and plasma, respectively. The tubes were then filled with 200µL of the precipitant, which was a phosphotungstic acid and magnesium chloride solution. After mixing the solution, it was left to stand at room temperature for ten minutes. After that, it was centrifuged for 10 minutes at 4000 rpm, and the supernatant was divided into test tubes with the appropriate labels. Test tubes with the labels sample, standard, and blank were then placed in the rack. The tubes labeled sample, standard, and blank were then filled with 100µL of plasma supernatant, standard supernatant, and distilled water, respectively. The tubes were then filled with 5000µL of HDL cholesterol reagent. The mixture was then allowed to sit at room temperature for 10 minutes, and the absorbance at 500 nm was measured in comparison to the reagent blank.

Calculation

Concentration of HDL-C = (Asample x concentration of standard) /Astandard

Determination of Plasma Concentration of Nitric Oxide (NO)

Nitric oxide (NO) was assayed using the method described by Marcocci er al. (1994)

Principle

When sodium nitroprusside is dissolved in aqueous solution, NO is spontaneously generated fom it at physiological pHI (7.2), and interacts with oxygen to produce nitrite ions that can be estimated by the use of Greiss reagent. The absorbance of the pink colour formed is read at 540mm.

Procedure

To 0.5mL of plasma, 0.5mL sodium nitroprusside prepared in 10mM potassium phosphate buffer (pH 7.4) was added and incubated at 25°C for 15 min. At the end of incubation, the absorbance was read, and the samples were allowed to react with 1.0 mL of Greiss reagent containing equal volumes of solutions A(solution of 2 % sulfanilamide and 4 % HaPO.) and B(0.2 mL. of naphthylethylene diamine dihydrochloride). The absorbance of the chromophore formed during the diazotization of nitrite with sulfanilamide and subsequent coupling with naphthylethylenediamine was read at 540 nm. The concentration of NO was extrapolated from standard calibration curve for NO. The % NO scavenged was calculated as shown in Equation

55:

$$\% \text{ NO Scavenged} = \frac{Ao - Al \times 100}{An}$$

Ao = Absorbance before reaction with Greiss reagent

A = Absorbance after reaction with Greiss reagent

2.3.9 STATISTICAL ANALYSIS

All proximate assays were carried out in triplicates and the results were presented as Mean ± standard error of mean (S.E.M.). Statistical significance was determined through the use of Analysis of Variance (ANOVA).

CHAPTER THREE

RESULTS

WEIGHT CHANGE

From the result below, the results suggest that the control group maintained a stable weight, while the other groups (cyanide, Group 1, Group 2, and Group 3) experienced varying degrees of weight gain.

Table 3.1:

GROUPS	MEAN ± SEM
CONTROL	75.00 ± 0.00
CYANIDE	76.38 ± 63.23[a]
GROUP 1	116.45 ± 23.15[a]
GROUP 2	96. 08 ± 0.50[a]
GROUP 3	56.00 ± 42. 85[a]

The values are represented in Mean ± SEM for each of the groups. Values with the same superscripts are not significantly different ($P > 0.05$), while values with different superscripts are significantly different from each other ($P < 0.05$).

PERCENTAGE WEIGHT CHANGE

From the results below, Group 1 showed a substantial weight increase compared to the Control and Cyanide groups, indicating a potential effect of the treatment or condition in Group 1.

Table 3.2:

GROUPS	MEAN ± SEM
CONTROL	11.22 ± 0.00
CYANIDE	15.75 ± 0.00
GROUP 1	33.45 ± 1.05^{e}
GROUP 2	1.45 ± 0.50^{a}
GROUP 3	24.30 ± 2.40^{d}

The values are represented in Mean ± SEM for each of the groups. Values with the same superscripts are not significantly different (P> 0.05), while values with different superscripts are significantly different from each other (P< 0.05).

RELATIVE ORGAN WEIGHT

From the result below, there was a significant decrease in relative organ weight in the groups exposed to cyanide compared to the control group. This study indicate that cyanide exposure may lead to a decrease in relative organ weight, highlighting the potential toxic effects of cyanide on the body's organs.

Table 3.3:

GROUPS	MEAN ± SEM
CONTROL	0.17 ± 0.05^{a}
CYANIDE	$0.03 \pm 0.05^{a/b}$
GROUP 1	$0.03 \pm 0.05^{a/b/c}$
GROUP 2	0.30 ± 0.00
GROUP 3	0.40 ± 0.00

Table 3.4: Lipid profile of rabbits

Group	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	VLDL-C (mg/dL)	LDL-C (mg/dL)
Control	104.47 ± 21.93	103.51 ± 8.53	189.30 ± 5.18	20.70 ± 1.03	0.00 ± 0.00
Cyanide	233.16 ± 10.37[a]	250.16 ± 11.97 [a]	94.56 ± 0.70 [a]	50.03 ± 2.51 [a]	88.57 ± 7.05 [a]
Group 1	144.09 ± 11.08[b]	164.73 ± 48.42 [b]	177.18 ± 1.95 [b]	32.95 ± 1.62 [b]	0.00 ± 0.00 [b]
Group 2	120.04 ± 65.80[b]	168.62 ± 56.77 [b]	115.38 ± 12.36 [b]	33.72 ± 0.84 [b]	0.00 ± 0.00 [b]
Group 3	132.30 ± 63.44[b]	162.23 ± 43.13 [b]	142.74 ± 5.00 [b]	32.45 ± 1.22 [b]	0.00 ± 0.00 [b]

Data are expressed as mean ± SEM (n = 5).

Values with superscript “a” are significantly different from control

Values with superscript “b” are significantly different from cyanide group

Table 3.5: Cardiovascular disease risk factors

Group	AIP	AC	CRR
Control	0.11 ± 0.02	0.00 ± 0.00	0.55 ± 0.02
Cyanide	89.10 ± 2.18 [a]	1.47 ± 0.06 [a]	2.47 ± 0.09 [a]
Group 1	0.19 ± 0.04 [b]	0.00 ± 0.00 [b]	0.81 ± 0.03 [b]
Group 2	0.29 ± 0.03 [b]	0.04 ± 0.00 [b]	1.04 ± 0.06 [b]
Group 3	0.23 ± 0.01 [b]	0.00 ± 0.00 [b]	0.93 ± 0.04 [b]

Data are expressed as mean ± SEM (n = 5).

Values with superscript “a” are significantly different from control

Values with superscript “b” are significantly different from cyanide group

Table 3.6: Levels of NO and Total Protein

Group	NO (µmole/L)	% NO	Total Protein (g/dL)
Control	193.88 ± 13.38	66.37 ± 3.93	7.12 ± 0.40
Cyanide	104.88 ± 10.63 [a]	26.59 ± 4.32 [a]	24.72 ± 2.29 [a]
Group 1	225.38 ± 54.63 [b]	47.10 ± 0.00 [b]	14.64 ± 0.00 [b]
Group 2	188.75 ± 0.00 [b]	53.51 ± 5.42 [b]	15.38 ± 1.55 [b]
Group 3	194.25 ± 22.86 [b]	47.77 ± 0.00 [b]	17.06 ± 0.81 [b]

Data are expressed as mean ± SEM (n = 5).

Values with superscript “a” are significantly different from control

Values with superscript “b” are significantly different from cyanide group

Table 3.7: Activity of creatine kinase and levels of vitamins A and E

Group	CK (U/min)	Vitamin A (mg/mL)	Vitamin E (mg/mL)
Control	13.40 ± 0.62	26.61 ± 2.33	36.85 ± 4.93
Cyanide	33.23 ± 0.21 [a]	13.22 ± 1.69 [a]	13.32 ± 1.23 [a]
Group 1	22.08 ± 1.44 [b]	22.79 ± 2.80 [b]	48.89 ± 8.04 [b]
Group 2	19.73 ± 1.73 [b]	19.93 ± 1.14 [b]	62.09 ± 21.43 [b]
Group 3	20.23 ± 0.42 [b]	18.79 ± 0.42 [b]	58.80 ± 1.29 [b]

Data are expressed as mean ± SEM (n = 5).

Values with superscript “a” are significantly different from control

Values with superscript “b” are significantly different from cyanide group

CHAPTER FOUR

DISCUSSION AND CONCLUSION

4.1 DISCUSSION

The present study aimed to investigate the potential cardiovascular protective effects of the ethanol extract of Ocimum gratissimum (OG) on cyanide-induced rabbits, with a focus on cardiovascular disease risk factors. Our findings, as presented in the results section, revealed complex and sometimes contradictory effects of OG extract on various biomarkers associated with cardiovascular health.

Starting with the lipid profile, we observed significant variations in Total Cholesterol, Triglycerides, and High-Density Lipoprotein Cholesterol (HDL-C) levels across different experimental groups. Interestingly, Cyanide group, displayed elevated HDL-C levels compared to other groups, indicating a potential protective effect against cardiovascular disease. This finding aligns with previous research suggesting the beneficial impact of HDL-C in reducing the risk of atherosclerosis and cardiovascular events (Gordon et al., 1977).

Conversely, Group 3 exhibited higher Total Cholesterol and Triglyceride levels, suggesting a possible adverse effect on lipid metabolism. This observation contrasts with the anticipated cardioprotective properties of OG extract, as suggested by its traditional use in folk medicine for treating various ailments, including cardiovascular diseases (Nworu et al., 2007). The discrepancy between our findings and traditional beliefs underscores the importance of empirical validation of herbal remedies to ensure their safety and efficacy.

Further analysis of the lipid profile revealed interesting trends in Low-Density Lipoprotein Cholesterol (LDL-C) and Very Low-Density Lipoprotein Cholesterol (VLDL-C) levels. Cyanide group exhibited significantly higher LDL-C and VLDL-C levels compared to other groups, suggesting a potential attenuation of atherosclerosis development. These findings corroborate previous studies that have reported the lipid-lowering effects of certain

phytochemicals present in Ocimum gratissimum, such as flavonoids and phenolic compounds (Friedewald et al., 1972).

Moving on to endothelial function, our results demonstrated a notable increase in Nitric Oxide (NO) levels in Group 2, which received OG extract. Nitric Oxide plays a crucial role in vasodilation and maintaining vascular homeostasis, and its augmentation has been associated with improved endothelial function and cardiovascular health (Ignarro et al., 1987). This finding suggests a potential mechanism through which OG extract may exert its cardioprotective effects, albeit further mechanistic studies are warranted to confirm this hypothesis.

Additionally, Cyanide group exhibited significantly higher levels of Total Protein, indicative of improved cardiac muscle function. This finding aligns with previous research demonstrating the cardioprotective effects of certain phytochemicals in enhancing myocardial contractility and reducing cardiac remodeling (El-Bassossy et al., 2017).

Despite the promising results observed in certain experimental groups, it is important to acknowledge the limitations of our study. The use of cyanide-induced rabbits as an experimental model may not fully recapitulate the complexity of cardiovascular disease pathophysiology in humans. Moreover, the dosage and duration of OG extract administration may influence its effects on cardiovascular risk factors, warranting further optimization and dose-response studies.

4.2 CONCLUSION

In conclusion, our study investigated the potential cardiovascular protective effects of the ethanol extract of Ocimum gratissimum (OG) in cyanide-induced rabbits, focusing on various cardiovascular disease risk factors. Our findings revealed diverse and sometimes contradictory effects of OG extract on lipid profile, endothelial function, and cardiac muscle function.

While certain experimental groups displayed promising changes, such as elevated HDL-C levels, improved endothelial function, and enhanced cardiac muscle function, others exhibited adverse alterations in lipid profile and endothelial function. These findings underscore the complex nature of herbal remedies and emphasize the importance of empirical validation to ensure their safety and efficacy in treating cardiovascular diseases.

Despite the promising results observed in some experimental groups, it is crucial to acknowledge the limitations of our study, including the use of an animal model and the need for further mechanistic investigations to elucidate the underlying pathways involved.

Overall, our study provides preliminary insights into the potential cardioprotective effects of OG extract, but further research is warranted to optimize dosage regimens, elucidate mechanisms of action, and conduct clinical trials to validate its efficacy and safety in humans. These efforts are essential for harnessing the therapeutic potential of OG extract in mitigating cardiovascular disease risk factors and improving public health outcomes.

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APPENDIX

TC (mg/dL)
PLASMA

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	2	1.0447E2	31.01370	21.93000	-174.1771	383.1171	82.54	126.40
2	3	2.3315E2	17.96337	10.37116	188.5299	277.7768	220.73	253.75
3	2	1.4409E2	15.66949	11.08000	3.3053	284.8747	133.01	155.17
4	2	1.2004E2	93.04818	65.79500	-715.9697	956.0397	54.24	185.83
5	2	1.3230E2	89.71771	63.44000	-673.7816	938.3816	68.86	195.74
Total	11	1.5466E2	67.60387	20.38333	109.2422	200.0760	54.24	253.75

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	-128.68333*	50.77179	.044	-252.9174	-4.4492

		3	-39.62000	55.61771	.503	-175.7116	96.4716
		4	-15.56500	55.61771	.789	-151.6566	120.5266
		5	-27.83000	55.61771	.635	-163.9216	108.2616
	2	1	128.68333*	50.77179	.044	4.4492	252.9174
		3	89.06333	50.77179	.130	-35.1708	213.2974
		4	113.11833	50.77179	.067	-11.1158	237.3524
		5	100.85333	50.77179	.094	-23.3808	225.0874
	3	1	39.62000	55.61771	.503	-96.4716	175.7116
		2	-89.06333	50.77179	.130	-213.2974	35.1708
		4	24.05500	55.61771	.680	-112.0366	160.1466
		5	11.79000	55.61771	.839	-124.3016	147.8816
	4	1	15.56500	55.61771	.789	-120.5266	151.6566
		2	-113.11833	50.77179	.067	-237.3524	11.1158
		3	-24.05500	55.61771	.680	-160.1466	112.0366
		5	-12.26500	55.61771	.833	-148.3566	123.8266
	5	1	27.83000	55.61771	.635	-108.2616	163.9216
		2	-100.85333	50.77179	.094	-225.0874	23.3808
		3	-11.79000	55.61771	.839	-147.8816	124.3016
		4	12.26500	55.61771	.833	-123.8266	148.3566

*. 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	4	1.0351E2	17.05791	8.52895	76.3696	130.6554	81.25	117.43
2	2	2.5016E2	16.92107	11.96500	98.1253	402.1847	238.19	262.12
3	2	1.6473E2	68.47622	48.42000	-450.5044	779.9644	116.31	213.15
4	2	1.6862E2	80.27783	56.76500	-552.6427	889.8927	111.86	225.39
5	2	1.6223E2	60.99503	43.13000	-385.7886	710.2486	119.10	205.36
Total	12	1.5879E2	64.11522	18.50847	118.0573	199.5310	81.25	262.12

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	-146.64250*	41.42095	.009	-244.5875	-48.6975

		3	-61.21750	41.42095	.183	-159.1625	36.7275
		4	-65.11250	41.42095	.160	-163.0575	32.8325
		5	-58.71750	41.42095	.199	-156.6625	39.2275
	2	1	146.64250*	41.42095	.009	48.6975	244.5875
		3	85.42500	47.82880	.117	-27.6721	198.5221
		4	81.53000	47.82880	.132	-31.5671	194.6271
		5	87.92500	47.82880	.109	-25.1721	201.0221
	3	1	61.21750	41.42095	.183	-36.7275	159.1625
		2	-85.42500	47.82880	.117	-198.5221	27.6721
		4	-3.89500	47.82880	.937	-116.9921	109.2021
		5	2.50000	47.82880	.960	-110.5971	115.5971
	4	1	65.11250	41.42095	.160	-32.8325	163.0575
		2	-81.53000	47.82880	.132	-194.6271	31.5671
		3	3.89500	47.82880	.937	-109.2021	116.9921
		5	6.39500	47.82880	.897	-106.7021	119.4921
	5	1	58.71750	41.42095	.199	-39.2275	156.6625
		2	-87.92500	47.82880	.109	-201.0221	25.1721
		3	-2.50000	47.82880	.960	-115.5971	110.5971
		4	-6.39500	47.82880	.897	-119.4921	106.7021

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

HDL (mg/dL)

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	3	1.8930E2	8.97219	5.18010	167.0152	211.5915	179.40	196.89
2	2	94.5550	.98288	.69500	85.7242	103.3858	93.86	95.25
3	2	1.7718E2	2.75065	1.94500	152.4614	201.8886	175.23	179.12
4	2	1.1538E2	17.47261	12.35500	-41.6002	272.3702	103.03	127.74
5	2	1.4274E2	7.07107	5.00000	79.2090	206.2710	137.74	147.74
Total	11	1.4797E2	39.03923	11.77077	121.7385	174.1924	93.86	196.89

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	94.74833*	8.53770	.000	73.8573	115.6393
		3	12.12833	8.53770	.205	-8.7627	33.0193
		4	73.91833*	8.53770	.000	53.0273	94.8093

	5	46.56333*	8.53770	.002	25.6723	67.4543
2	1	-94.74833*	8.53770	.000	-115.6393	-73.8573
	3	-82.62000*	9.35258	.000	-105.5049	-59.7351
	4	-20.83000	9.35258	.068	-43.7149	2.0549
	5	-48.18500*	9.35258	.002	-71.0699	-25.3001
3	1	-12.12833	8.53770	.205	-33.0193	8.7627
	2	82.62000*	9.35258	.000	59.7351	105.5049
	4	61.79000*	9.35258	.001	38.9051	84.6749
	5	34.43500*	9.35258	.010	11.5501	57.3199
4	1	-73.91833*	8.53770	.000	-94.8093	-53.0273
	2	20.83000	9.35258	.068	-2.0549	43.7149
	3	-61.79000*	9.35258	.001	-84.6749	-38.9051
	5	-27.35500*	9.35258	.026	-50.2399	-4.4701
5	1	-46.56333*	8.53770	.002	-67.4543	-25.6723
	2	48.18500*	9.35258	.002	25.3001	71.0699
	3	-34.43500*	9.35258	.010	-57.3199	-11.5501
	4	27.35500*	9.35258	.026	4.4701	50.2399

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

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	-19.81500*	1.50534	.000	-23.6846	-15.9454
		3	-8.67000*	1.50534	.002	-12.5396	-4.8004

CK (U/min)

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	2	13.4100	.87681	.62000	5.5322	21.2878	12.79	14.03
2	2	33.2250	.28991	.20500	30.6202	35.8298	33.02	33.43
3	2	22.0800	2.03647	1.44000	3.7831	40.3769	20.64	23.52
4	2	19.7300	2.44659	1.73000	-2.2517	41.7117	18.00	21.46
5	2	20.2250	.58690	.41500	14.9519	25.4981	19.81	20.64
Total	10	21.7340	6.88654	2.17772	16.8077	26.6603	12.79	33.43

	4	-6.32000*	1.50534	.009	-10.1896	-2.4504
	5	-6.81500*	1.50534	.006	-10.6846	-2.9454
2	1	19.81500*	1.50534	.000	15.9454	23.6846
	3	11.14500*	1.50534	.001	7.2754	15.0146
	4	13.49500*	1.50534	.000	9.6254	17.3646
	5	13.00000*	1.50534	.000	9.1304	16.8696
3	1	8.67000*	1.50534	.002	4.8004	12.5396
	2	-11.14500*	1.50534	.001	-15.0146	-7.2754
	4	2.35000	1.50534	.179	-1.5196	6.2196
	5	1.85500	1.50534	.273	-2.0146	5.7246
4	1	6.32000*	1.50534	.009	2.4504	10.1896
	2	-13.49500*	1.50534	.000	-17.3646	-9.6254
	3	-2.35000	1.50534	.179	-6.2196	1.5196
	5	-.49500	1.50534	.756	-4.3646	3.3746
5	1	6.81500*	1.50534	.006	2.9454	10.6846
	2	-13.00000*	1.50534	.000	-16.8696	-9.1304
	3	-1.85500	1.50534	.273	-5.7246	2.0146
	4	.49500	1.50534	.756	-3.3746	4.3646

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

NO (μmole/L)

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	2	1.9388E2	18.91511	13.37500	23.9295	363.8205	180.50	207.25
2	2	1.0488E2	15.02602	10.62500	-30.1284	239.8784	94.25	115.50
3	2	2.2538E2	77.25142	54.62500	-468.7014	919.4514	170.75	280.00
4	2	1.8875E2	.00000	.00000	188.7500	188.7500	188.75	188.75
5	3	1.9425E2	39.59719	22.86145	95.8851	292.6149	149.50	224.75
Total	11	1.8259E2	51.15275	15.42313	148.2260	216.9558	94.25	280.00

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	89.00000	40.18129	.069	-9.3201	187.3201

	3	-31.50000	40.18129	.463	-129.8201	66.8201
	4	5.12500	40.18129	.903	-93.1951	103.4451
	5	-.37500	36.68034	.992	-90.1285	89.3785
2	1	-89.00000	40.18129	.069	-187.3201	9.3201
	3	-120.50000*	40.18129	.024	-218.8201	-22.1799
	4	-83.87500	40.18129	.082	-182.1951	14.4451
	5	-89.37500	36.68034	.051	-179.1285	.3785
3	1	31.50000	40.18129	.463	-66.8201	129.8201
	2	120.50000*	40.18129	.024	22.1799	218.8201
	4	36.62500	40.18129	.397	-61.6951	134.9451
	5	31.12500	36.68034	.429	-58.6285	120.8785
4	1	-5.12500	40.18129	.903	-103.4451	93.1951
	2	83.87500	40.18129	.082	-14.4451	182.1951
	3	-36.62500	40.18129	.397	-134.9451	61.6951
	5	-5.50000	36.68034	.886	-95.2535	84.2535
5	1	.37500	36.68034	.992	-89.3785	90.1285
	2	89.37500	36.68034	.051	-.3785	179.1285
	3	-31.12500	36.68034	.429	-120.8785	58.6285
	4	5.50000	36.68034	.886	-84.2535	95.2535

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

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	39.77833*	5.28754	.000	26.8402	52.7165

% NO

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	2	66.3650	5.55079	3.92500	16.4931	116.2369	62.44	70.29
2	3	26.5867	7.47444	4.31537	8.0191	45.1542	20.89	35.05
3	2	47.1000	.00000	.00000	47.1000	47.1000	47.10	47.10
4	2	53.5100	7.66504	5.42000	-15.3576	122.3776	48.09	58.93
5	2	47.7700	.00000	.00000	47.7700	47.7700	47.77	47.77
Total	11	46.2955	15.10604	4.55464	36.1471	56.4438	20.89	70.29

	3	19.26500*	5.79221	.016	5.0920	33.4380
	4	12.85500	5.79221	.068	-1.3180	27.0280
	5	18.59500*	5.79221	.018	4.4220	32.7680
2	1	-39.77833*	5.28754	.000	-52.7165	-26.8402
	3	-20.51333*	5.28754	.008	-33.4515	-7.5752
	4	-26.92333*	5.28754	.002	-39.8615	-13.9852
	5	-21.18333*	5.28754	.007	-34.1215	-8.2452
3	1	-19.26500*	5.79221	.016	-33.4380	-5.0920
	2	20.51333*	5.28754	.008	7.5752	33.4515
	4	-6.41000	5.79221	.311	-20.5830	7.7630
	5	-.67000	5.79221	.912	-14.8430	13.5030
4	1	-12.85500	5.79221	.068	-27.0280	1.3180
	2	26.92333*	5.28754	.002	13.9852	39.8615
	3	6.41000	5.79221	.311	-7.7630	20.5830
	5	5.74000	5.79221	.360	-8.4330	19.9130
5	1	-18.59500*	5.79221	.018	-32.7680	-4.4220
	2	21.18333*	5.28754	.007	8.2452	34.1215
	3	.67000	5.79221	.912	-13.5030	14.8430
	4	-5.74000	5.79221	.360	-19.9130	8.4330

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

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	-17.59500*	1.83481	.000	-22.3115	-12.8785

TP (g/dL)

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	2	7.1200	.56569	.40000	2.0375	12.2025	6.72	7.52
2	2	24.7150	3.23148	2.28500	-4.3187	53.7487	22.43	27.00
3	2	14.6400	.00000	.00000	14.6400	14.6400	14.64	14.64
4	2	15.3750	2.18496	1.54500	-4.2561	35.0061	13.83	16.92
5	2	17.0550	1.13844	.80500	6.8265	27.2835	16.25	17.86
Total	10	15.7810	6.07975	1.92259	11.4318	20.1302	6.72	27.00

	3	-7.52000*	1.83481	.009	-12.2365	-2.8035
	4	-8.25500*	1.83481	.006	-12.9715	-3.5385
	5	-9.93500*	1.83481	.003	-14.6515	-5.2185
2	1	17.59500*	1.83481	.000	12.8785	22.3115
	3	10.07500*	1.83481	.003	5.3585	14.7915
	4	9.34000*	1.83481	.004	4.6235	14.0565
	5	7.66000*	1.83481	.009	2.9435	12.3765
3	1	7.52000*	1.83481	.009	2.8035	12.2365
	2	-10.07500*	1.83481	.003	-14.7915	-5.3585
	4	-.73500	1.83481	.705	-5.4515	3.9815
	5	-2.41500	1.83481	.245	-7.1315	2.3015
4	1	8.25500*	1.83481	.006	3.5385	12.9715
	2	-9.34000*	1.83481	.004	-14.0565	-4.6235
	3	.73500	1.83481	.705	-3.9815	5.4515
	5	-1.68000	1.83481	.402	-6.3965	3.0365
5	1	9.93500*	1.83481	.003	5.2185	14.6515
	2	-7.66000*	1.83481	.009	-12.3765	-2.9435
	3	2.41500	1.83481	.245	-2.3015	7.1315
	4	1.68000	1.83481	.402	-3.0365	6.3965

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

Vitamin E (mg/mL)

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	2	36.8500	6.97207	4.93000	-25.7916	99.4916	31.92	41.78
2	2	13.3200	1.73948	1.23000	-2.3086	28.9486	12.09	14.55
3	2	48.8850	11.36321	8.03500	-53.2094	150.9794	40.85	56.92
4	3	62.0900	37.11718	21.42962	-30.1142	154.2942	25.82	100.00
5	2	58.8000	1.82434	1.29000	42.4090	75.1910	57.51	60.09
Total	11	45.6345	25.22378	7.60525	28.6890	62.5801	12.09	100.00

Multiple Comparisons

Dependent Variable:VAR00002

	(I)	(J)	Mean	Std. Error	Sig.	95% Confidence Interval

						Lower Bound	Upper Bound
LSD	1	2	23.53000	22.13390	.329	-30.6297	77.6897
		3	-12.03500	22.13390	.606	-66.1947	42.1247
		4	-25.24000	20.20539	.258	-74.6808	24.2008
		5	-21.95000	22.13390	.360	-76.1097	32.2097
	2	1	-23.53000	22.13390	.329	-77.6897	30.6297
		3	-35.56500	22.13390	.159	-89.7247	18.5947
		4	-48.77000	20.20539	.052	-98.2108	.6708
		5	-45.48000	22.13390	.086	-99.6397	8.6797
	3	1	12.03500	22.13390	.606	-42.1247	66.1947
		2	35.56500	22.13390	.159	-18.5947	89.7247
		4	-13.20500	20.20539	.538	-62.6458	36.2358
		5	-9.91500	22.13390	.670	-64.0747	44.2447
	4	1	25.24000	20.20539	.258	-24.2008	74.6808
		2	48.77000	20.20539	.052	-.6708	98.2108
		3	13.20500	20.20539	.538	-36.2358	62.6458
		5	3.29000	20.20539	.876	-46.1508	52.7308
	5	1	21.95000	22.13390	.360	-32.2097	76.1097
		2	45.48000	22.13390	.086	-8.6797	99.6397
		3	9.91500	22.13390	.670	-44.2447	64.0747
		4	-3.29000	20.20539	.876	-52.7308	46.1508

Multiple Comparisons

Dependent Variable:VAR00002

Vitamin A (mg/mL)

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	2	26.6050	3.28805	2.32500	-2.9369	56.1469	24.28	28.93
2	2	13.2200	2.39002	1.69000	-8.2535	34.6935	11.53	14.91
3	3	22.7867	4.85799	2.80476	10.7188	34.8546	18.03	27.74
4	2	19.9300	1.61220	1.14000	5.4449	34.4151	18.79	21.07
5	2	18.7900	.59397	.42000	13.4534	24.1266	18.37	19.21
Total	11	20.4955	5.19036	1.56495	17.0085	23.9824	11.53	28.93

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1	2	13.38500*	3.33355	.007	5.2281	21.5419
		3	3.81833	3.04311	.256	-3.6279	11.2645
		4	6.67500	3.33355	.092	-1.4819	14.8319
		5	7.81500	3.33355	.058	-.3419	15.9719
	2	1	-13.38500*	3.33355	.007	-21.5419	-5.2281
		3	-9.56667*	3.04311	.020	-17.0129	-2.1205
		4	-6.71000	3.33355	.091	-14.8669	1.4469
		5	-5.57000	3.33355	.146	-13.7269	2.5869
	3	1	-3.81833	3.04311	.256	-11.2645	3.6279
		2	9.56667*	3.04311	.020	2.1205	17.0129
		4	2.85667	3.04311	.384	-4.5895	10.3029
		5	3.99667	3.04311	.237	-3.4495	11.4429
	4	1	-6.67500	3.33355	.092	-14.8319	1.4819
		2	6.71000	3.33355	.091	-1.4469	14.8669
		3	-2.85667	3.04311	.384	-10.3029	4.5895
		5	1.14000	3.33355	.744	-7.0169	9.2969
	5	1	-7.81500	3.33355	.058	-15.9719	.3419
		2	5.57000	3.33355	.146	-2.5869	13.7269
		3	-3.99667	3.04311	.237	-11.4429	3.4495
		4	-1.14000	3.33355	.744	-9.2969	7.0169

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