EFFECTS OF RECOMMENDED DRUGS USED IN THE TREATMENT OF COVID-19 INFECTION ON LIPID PROFILE IN ALBINO WISTAR RATS

BY

UNUKPO, BLESSING
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BENIN CITY.

THIS PROJECT IS SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, UNIVERSITY OF BENIN IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF MEDICAL LABORATORY SCIENCE DEGREE

APRIL, 2024.

CERTIFICATION

This is to certify that this project work was carried out by **BLESSING UNUKPO (F),** with matriculation number **BMS1802499** in the Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, University of Benin (UNIBEN), under the supervision of **MR. O.F. AMEGOR** in partial fulfillment of the requirement of Bachelor of Medical Laboratory Science Degree.

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(HEAD OF DEPARTMENT)	
_____	_____
EXTERNAL EXAMINER	**DATE**

DEDICATION

I dedicate this project work to Jehovah God Almighty for the wisdom and inspiration it took to bring this seminar work to reality. I also dedicate this seminar work to my parents, Mr. and Mrs. Ovoke Unukpo for being my pillar of support through it all. Additionally, I dedicate this project work to my siblings, Bravo Unukpo and Gift Unukpo for their support too.

Finally, I dedicate this project work to all Medical Laboratory Science Students and healthcare professionals both in the academic sector and clinical sector who are working so hard every day and every night to bring out the best medical therapeutic practices in the medical field.

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To my bestie Eghaghe Peculiar, my dear friend Comfort Solomon, my big bro Ereyimwen Osakpawman Augustine, Med. Lab. Scientist Victor Okonkwo and his gorgeous wife, Med. Lab. Scientist Mrs. Erhabor Ikponmwosa, Miss Paulina A.J., Med. Lab. Scientist Blessing Ayeni, Med. Lab. Scientist Ukpe Michael Pius, my dear friends: Aondokume Nguamo Felicia, Adewale Priscilla Shalom, Stephaine Osahumen Osazuwa, thanks for being present through the thick and thin moments, you all are loved!

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ABSTRACT

The SARS-CoV-2 virus is the infectious agent that causes COVID-19, commonly referred to as coronavirus illness. Chloroquine (CQ), Hydroxylchloroquine (HCQ), Lopinavor/Retinavir (L/R), and other medications were tested for treating COVID-19 infection. Alcohol or phosphate functional group molecules are uncommon in lipids, which are esters of fatty acids that are soluble in organic solvents but insoluble in water. Triglycerides, HDL cholesterol, LDL cholesterol, and total cholesterol make up the lipid profile. The aim of the thesis is to ascertain and assess how prescribed medications for the management of COVID-19 infection affect albino rats' lipid levels. In total, 60 albino wistar rats were employed in this investigatigation. 6 were used as negative controls (given with water and feed only), while 54 were used as positive controls (administered with the medications). On the 29th day, blood samples were collected from the albino wistar rats into plain containers and serum were obtained for laboratory analysis of the lipid profile indices.. Version 27 of SPSS (Statistical Package for Social Sciences) was used to analyze the study's data. For both tests and controls, the Standard Error of Mean was expressed as mean ± S.E.M. Additionally, an ANOVA was used to compare the results at a 95% confidence interval ($P<0.05$). Notably, the combination treatment containing Hydroxylchloroquine (HCQ), Ivermectin (IV), L/R (Lopinavir/Retinavir), Azithromycin (AZI), Zinc (Zn), and Selenium (Se) led to a significant decrease in HDL_c levels ($P<0.05$) and weight ($P<0.05$) compared to the control group. This was in addition to the notable reduction in total cholesterol (TC) levels ($P <0.05$) that HCQ showed. Additionally, compared to the control group, Chloroquine (CQ) showed significantly lower cardiac risk ratios ($P<0.05$) and atherogenic coefficients ($P<0.05$), suggesting a possible decrease in cardiovascular risk and atherogenic potential. Additionally, compared to the control group, the Hydroxychloroquine (HCQ) treatment group showed significantly decreased cardiac risk ratios ($P<0.05$) and atherogenic coefficients ($P<0.05$). Nevertheless, the combination therapy with CQ, IV, L/R, AZI, Zn, and Se showed noticeably greater atherogenic coefficients and cardiac risk ratios. In conclusion, the study elucidated the various effects of the drugs on lipid profile, weight, cardiac risk ratio, and atherogenic coefficient. However, while both CQ and HCQ treatments led to significant weight gain, contrary to some findings, their mechanisms of action on weight regulation remain complex and warrant further investigation.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

In December 2019, novel coronavirus disease (COVID-19) emerged in Wuhan, China, attracting the notice of regional authorities and rapidly drawing global attention. According to the WHO, in less than 4 months, COVID-19 spread through almost all countries and regions. The COVID-19 pandemic was wreaking havoc on the world economy, in addition to creating the current global health crisis (Momtazmanesh *et al.,* 2020).

Six stages in the SARS-CoV-2 life cycle have been identified by research: (1) adhesion and entry; (2) uncoating; (3) directing gRNA replication; (4) translation within the Golgi apparatus and endoplasmic reticulum; (5) assembly; and (6) release of virion (Pitlik, 2020).

Over the past two decades, the emergence of coronavirus-associated diseases (SARS and MERS) inflicted global challenges to public health systems. SARS-CoV-2 (the causative agent for coronavirus disease COVID-19) is the latest addition to this growing list of unwelcomed novel agents. The WHO declared COVID-19 a public health emergency of international concern on 30 January and a pandemic on 11 March 2020 (Balkhair, 2020).

Numerous accounts have included headaches, dizziness, sleeplessness, palpitations, and persistent exhaustion along with bone and joint discomfort. In cases of severe

lung illness, additional symptoms were observed, suggesting irreversible pulmonary scarring and dysfunction (El-Shabasy *et al., 2*022).

Tests are being conducted on about fifteen different medications to treat COVID-19 infections. Among these include ivermectin, lopinavir and ritonavir, nafamostat and camostat, famotidine, umifenovir, nitazoxanide, corticosteroids, bevacizumab, fluvoxamine, and tocilizumab and sarilumab (Khan et al., 2020). Remdesivir is a possible medication for COVID-19 therapy. Adenosine C-nucleoside and broad-spectrum antiviral agent, it is phosphoramidate prodrug. A protease inhibitor that has a high selectivity for HIV-1 protease is called lipinavir. By interfering with bacteria's ability to synthesize proteins, azithromycin stops them from developing. It binds to the bacterial ribosome's 50S subunit, preventing mRNA translation. A strong anthelmintic medication is ivermectin **(**Wu *et al.,* 2020).

Lipids are organic compounds that are insoluble in water and soluble in organic solvents. They are esters of fatty acids, rarely containing alcohol or phosphate functional group molecules, and comprise triglycerides, phospholipids, and steroids. They are the energy reserves of animals and perform various functions, such as maintenance of body temperature, whilst being the key constituents of cell membranes and serving as chemical messengers. The human body requires various types of useful lipid fat to maintain the healthy functions of its parts. Maintaining blood lipid balance is critical to overall health. Fat deposits in artery walls are a result of abnormal blood lipid levels, and this leads to problems inside the blood arteries. Diabetes, alcoholism, renal disease, hypothyroidism, liver disease, and stress are among the factors that contribute

to high lipid levels. Increased lipids readily attach themselves to the blood's circulating nerve walls, leading to an increase in fatty scale and a host of atherosclerosis conditions, including heart attacks and strokes (Natesan and Kim, 2021).

Glycocans are typically present on cells as glycoproteins or glycolipids, where they are covalently connected to lipids or proteins, respectively. Instead of analyzing the glycoprotein as a whole, most glycoprotein analyses are top-down, in which case the glycoprotein is broken down into smaller pieces (Shajahan et *al.,* 2017).

- LDL is directly atherogenic because its small size allows it to infiltrate into the walls of inflamed vascular endothelium (Each 1mg/dL increase in LDL increases the risk of a coronary artery event by 1%-1.5%). Normally LDL is not irritating to the tissues but when it lingers too long in the oxygen rich subendothelium, its lipids become peroxidized whereupon the LDL particle is attracted to the scavenger receptor of a nearby tissue macrophage & is ingested.(Hastings, 2005).

- High Density Lipoprotein (HDL) conveys cholesterol back to the liver. HDL contains Apo-A lipoproteins and is antiatherogenic (every 1mg/dL increase in plasma HDL confers a 1%-1.5% decrease in the probability of a coronary artery event) (Hastings, 2005).

- VLDL & IDL, if present in excessive quantities may be indirectly atherogenic. The reason is that both have about 20 times stronger an affinity for the LDL receptor than does LDL (That’s because both VLDL & IDL contain apo-E, which confers this higher affinity, whereas LDL does not) (Hastings, 2005).

Triglycerides, LDL cholesterol, HDL cholesterol, and total cholesterol are all included in the plasma lipid profile (Vasudevan *et al.*, 2011).

1.2 Statement of Problem

It has been demonstrated that using prescribed medications to treat COVID-19 infection can have an impact on the cardiovascular system, including alterations in cholesterol levels. On the other hand, not much is known about how these medications specifically affect the level of the lipid profile, particularly when used in combination therapy. Therefore, it is important to investigate the effects of recommended drugs on lipid profile in order to understand their potential implications for patients health. This study will help to fill the gap in knowledge on the effects of specific recommended drugs on lipid profile, atherogenic indices, and weight.

1.3 Justification of Study

Impaired lipid profile levels relates so well to the development of cardiovascular diseases. In the advent of COVID-19 infection, recommended drugs are used for management and treatment

Moreover, these drugs have their primary usage to treat other specific infections. But, the use of recommended anti-COVID-19 drugs in combination has not fully been established. Hence, the adverse effects in drug combination with specific interest to lipids in albino rats is of special interest in the study of drug reactions.

However, the focus of this study is on the drugs itself, as the albino rats were not exposed to covid-19 during this research. Therefore, the results of this study can give a valuable insight for further research studies when analyzed with positive covid-19 specimens.

1.4 Aim of Study

To evaluate the effects of recommended drugs used for the treatment of COVID-19 infection on lipid profile in albino rats.

1.5 Objectives of Study

i) To evaluate the effects of recommended drugs used for the treatment of COVID-19 infection on triglycerides in albino rats.

ii) To evaluate the effects of recommended drugs used for the treatment of COVID-19 infection on high density lipoprotein cholesterol in albino rats.

iii) To evaluate the effects of recommended drugs used for the treatment of COVID-19 infection on low density lipoprotein cholesterol in albino rats.

iv) To evaluate the effects of recommended drugs used for the treatment of COVID-19 infection on total cholesterol in albino rats.

1.6 Research Questions

1) What notable impacts does Ivermectin (IV), Hydroxylchloroquine (HCQ), Ivermectin (IV), Lopinavir/Retinavir (L/R), Azithromycin (AZI), Zinc (Zn), and Selinum (Se) have on the albino wistar rats' lipid profiles?
2) What notable impacts do the medications (in combination therapy) have on the albino wistar rats' lipid profiles?
3) Are there effects on the atherogenic indices of lipids in the albino wistar rats?
4) Does the drugs have effect on the weight of the albino wistar rats?

CHAPTER TWO

LITERATURE REVIEW

2.1 The First Human Coronavirus

June Dalziel Almeida published a description of the first human coronavirus in 1966. She had been working on a study looking at the causes of the common cold when she saw a viral structure visible under an electron microscope (Pitlik, 2020).

Interestingly, COVID-19 patients' symptoms, epidemiology, incubation period, and radiological results are nearly identical to those of SARS patients. Usually, the engagement of receptors on the surface of the host cell membrane results in coronavirus infections. It was eventually shown that SARS-CoV enters the host cell by means of pH- and receptor-mediated endocytosis, rather than the original theory of direct fusion with the plasma membrane. The coronavirus uses clathrin-mediated endocytosis to enter the host cell. During this process, the coronavirus's spike glycoproteins, or S proteins, bind to receptors on the host cell. These structural proteins are necessary for the coronavirus, which is made up of the S1 and S2 subunits, to assemble and infect. The cellular receptor is bound by the S1 subunit, which has the receptor-binding domain (RBD), while the S2 subunit helps with the fusion and entry process (Khan *et al.*, 2020).

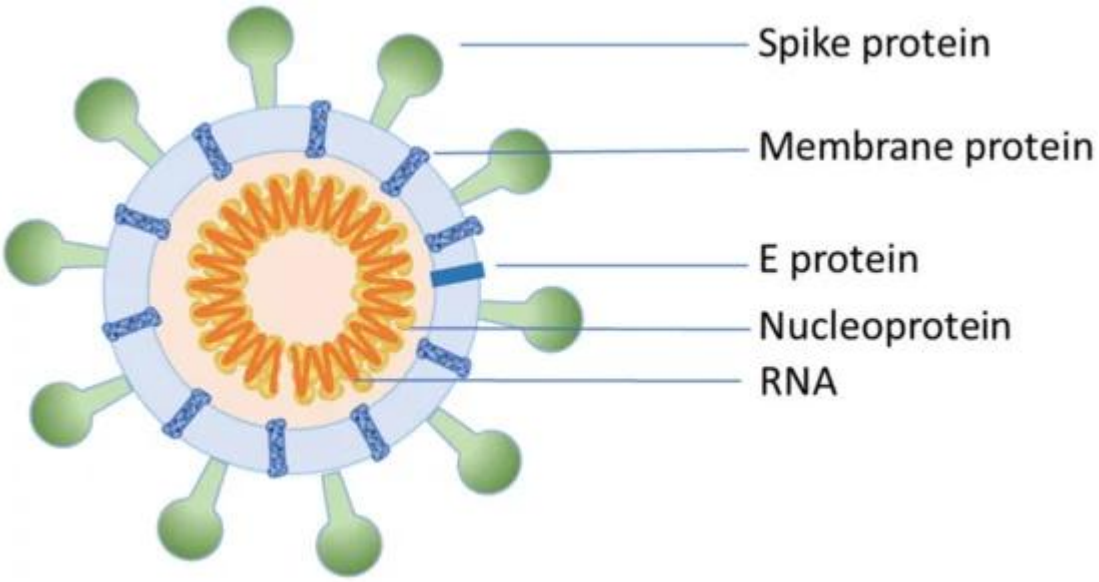

Fig. 2.1: Typical scheme of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virion structure (Khan *et al.*, 2020).

2.2 Epidemiology

Since the outbreak of COVID-19 in December in Wuhan, China, the infection has rapidly spread to other parts of the world, and the growing number of cases clearly suggests that the illness is still continuously spreading. Initially, several cases (>50 people) of acute pneumonia associated with COVID-19 were reported in China, which were linked to a seafood market in the Wuhan province. Since then, the number of infected individuals has reached around ten million, which may still be an underestimate since there is a strong possibility of untraced exposures and asymptotic individuals. Sequence-based analysis of isolates from patients has led to the identification of the causative agent as a novel form of coronavirus. Besides, sequencing technology and other techniques have significantly helped in correct diagnosis of the viral infection (Khan *et al.*, 2020)**.**

2.3 Symptoms of COVID-19

Several reports have included headaches, palpitations, dizziness, sleeplessness, and bone and joint pain in addition to ongoing exhaustion. In patients with severe pulmonary illness, additional signs of irreversible lung scarring and dysfunction have been observed (El-Shabasy *et al., 2*022).

On the other hand, smoking, advanced age, and medical comorbidities, such as diabetes mellitus, hypertension, and obesity, are risk factors for severe SARS-COV-2 infection and rising rates of mortality (El-Shabasy *et al., 2*022).

2.4 Treatment of COVID-19

(A) **Drugs:** Around 15 different drugs are being tested for the treatment of COVID-19 infections. These include, chloroquine and hydroxychloroquine, lopinavir and ritonavir, nafamostat and camostat, famotidine, umifenovir, nitazoxanide, ivermectin, corticosteroids, tocilizumab and sarilumab, bevacizumab, and fluvoxamine. Various antiviral agents such as remdesivir and ribavirin were tested for their efficacy in treating the disease (Khan *et al.*, 2020).

(B) **Support therapy/management:** These include therapies such as artificial liver system (ALS) and extracorporeal membrane oxygenation (ECMO). It is clear from various trials and other studies that there is no specific treatment for COVID-19 and the patients are being treated using combinations of different medicines and management practices (Khan *et al.*, 2020).

2.5 Examples of Drugs Recommended for the Treatment of COVID-19

2.5.0 Remdesivir

Remdesivir is a potential drug for treatment of COVID-19. It is a phosphoramidate prodrug of an adenosine C-nucleoside and a broad-spectrum antiviral agent synthesized and developed by Gilead Sciences in 2017 as a treatment for Ebola virus infection. Remdesivir is metabolized into its active form, GS-441524, that obscures viral RNA polymerase and evades proofreading by viral exonuclease, causing a decrease in viral RNA production. The antiviral mechanism of remdesivir is a delayed chain cessation of nascent viral RNA **(**Wu *et al.*, 2020).

2.5.1 Hydroxychloroquine and Chloroquine

Chloroquine can block the entry of SARS-CoV-2 and stop virus-cell fusion by interfering with the glycosylation of the ACE2 receptor and its interaction with spike protein. This shows that early in the infection process, before COVID-19 diminishes ACE2 expression and activity, therapy with chloroquine may be more beneficial. Regarding Th17-related cytokines (IL-6, IL-17, and IL-22), hydroxychloroquine possesses anti-inflammatory properties that are observed in patients with systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) as well as in healthy individuals **(**Wu *et al.*, 2020).

2.5.2 Lopinavir-Ritonavir

Protease inhibitor lopinavir has a high selectivity for HIV-1 protease. Ritonavir is the only medication that is sold and used in conjunction with lopinavir. Abbott initially

introduced this combination to the market in 2000 under the Kaletra brand. Owing to lopinavir's high biotransformation and poor oral bioavailability, ritonavir and lopinavir are coformulated to increase exposure. Ritonavir "boosts" lopinavir exposure and enhances antiviral activity since it is a strong inhibitor of the enzymes that are in charge of lopinavir metabolism. Lopinavir is a peptidomimetic compound with a hydroxyethylene scaffold that resembles the peptide linkage that the HIV-1 protease enzyme normally targets. Because the scaffold cannot split on its own, it inhibits the activity of the HIV-1 protease **(**Wu *et al.,* 2020).

2.5.3 Azithromycin

Antibiotics such as azithromycin can be used to treat a wide range of infections, including skin infections, lung infections, and STDs due to susceptible bacteria. Furthermore, it has been demonstrated to be effective in vitro against the Zika and Ebola viruses and to shield individuals with viral infections from developing serious respiratory tract infections. Azithromycin works by interfering with the synthesis of proteins in bacteria, so preventing them from multiplying. It inhibits mRNA translation by attaching to the bacterial ribosome's 50S subunit **(**Wu *et al.,* 2020).

2.5.4 Ibuprofen

Like ACE inhibitors or ARBs, some anti-inflammatory medications, such as ibuprofen, a nonsteroidal anti-inflammatory medicine (NSAID), activate ACE2 receptors. Using them may raise your chance of getting COVID-19. Ibuprofen might not be hazardous because SARS-CoV infections can be prevented from causing catastrophic lung failure by blocking the renin-angiotensin pathway **(**Wu *et al.,* 2020).

2.5.5 Ivermectin

It was initially shown that the powerful anthelmintic medication ivermectin inhibited the interaction between the HIV-1 integrase (IN) protein and its nuclear transport receptor importin α/β. Additional research demonstrates that it has the ability to stop the spread of many other viruses, such as the influenza, flavivirus, and dengue virus.Ivermectin has recently demonstrated up to 5000-fold suppression against SARS-CoV-2 at 48 hours in vitro. Its antiviral effect is likely due to inhibiting IMPα/β1-mediated nuclear import of viral proteins. Finding out how it inhibits SARS-CoV-2 in vivo will be intriguing **(**Wu *et al.*, 2020).

2.6 Subchronic Administration of Anti-COVID-19 drugs: Subchronic oral toxicity testing (repeated dose 90-day oral toxicity testing)

To investigate a substance's subchronic toxicity, both rats and non-rodents are used. For ninety days, the test drug is taken orally. During that time, behavioral, biochemical, and cardiovascular parameter changes, weekly fluctuations in body weight, and monthly changes are noted. The study's experimental animals are killed at the conclusion. Histopathological examinations are performed on all the tissues, and gross pathology alterations are noted. The range of weight variation allowed is ±20%, and there should be minimal individual variance among the animals. The study procedure may include a satellite group that consists of a high-dose group and a control group **(**Parasuraman, 2011).

2.7 Some Recommended Anti-COVID-19 Drugs

2.7.0 Chloroquine

The chemical compound chloroquine is 7-chloro-4(4-diethylamino-1-methylbutylamino) quinoline; its hydroxyl derivative is called hydroxychloroquine. The molecular weights of hydroxychloroquine (336) and chloroquine (320) are different. Based on two fused aromatic rings with conjugated double bonds, the 4-aminoquinoline nucleus, chloroquine and hydroxychloroquine are both amphiphilic weak bases. Each medicine has good cell membrane penetration. In addition to being less lipophilic and more polar, hydroxychloroquine has greater trouble diffusing through cell membranes. The third benzene ring, which forms part of quinacrine's acridine nucleus, is absent from the 4AQs. Chloroquine forms hydrogen bonds, van der Waals forces, and electrostatic interactions with nucleic acids. It binds ionically to melanin, a polyanion with many negatively charged carboxyl groups and ortho-semiquinone groups, as a cation at physiologic pH. Van der Waals forces between the indole nuclei of melanin and the aromatic rings of chloroquine, as well as charge transfer complexes in which melanin serves as an electron acceptor, are additional binding forces to melanin. Melanin and hydroxychloroquine interact similarly to chloroquines (Browning and Browning, 2014).

2.7.1 Hydroxylchloroquine

Zhou *et al* demonstrated that hydroxychloroquine could provide better outcomes than chloroquine for the treatment of SARS-CoV-2 infection. The authors highlighted three likely mechanisms for how these two drugs are beneficial for protecting from the

development of and complications from COVID-19 virus infections: (1) inhibition of receptor binding by the virus; (2) inhibition of membrane fusion by the virus; and (3) immune modulation to decrease cytokine release. Hydroxychloroquine appears to decrease the dangerous progression of COVID-19 toward cytokine storm by reducing CD154 expression in T-cells. Moreover, the authors suggested that hydroxychloroquine, compared to chloroquine, has fewer side effects, and is more potent at maximum tolerated doses. In the end, consensus reports were released by specialists 2020–22 through multidisciplinary cooperation with the Guangdong Province Department of Science and Technology, the Guangdong Consensus Health Commission, and the State Council of China. For patients with mild, moderate, and severe instances of new coronavirus pneumonia who did not have a contraindication to chloroquine, these expert panels suggested 500 mg tablets of chloroquine phosphate twice daily for ten days (Meo *et al.*, 2020).

2.7.2 Azithromycin

Azithromycin presents *in vitro* activity against SARS-CoV-2 and could act in different points of the viral cycle. Its immunomodulatory properties include the ability to downregulate cytokine production, maintain epithelial cell integrity or prevent lung fibrosis. Azithromycin use was associated with a reduction in mortality and ventilation days in other viral infections. These properties could be beneficial throughout the COVID-19. However, the evidence of its use is scarce and of low quality. Azithromycin has been assessed in retrospective observational studies mainly in combination with hydroxychloroquine, which has shown to provide no benefit. This

macrolide presents a well-known safety profile. Upcoming clinical trials will determine the role of azithromycin in the COVID-19 (including the stage of the disease where it offers the greatest benefits and the effect of its combination with other drugs) (Echeverría-Esnal *et al.*, 2021).

Known by the brand name Zithromax, azithromycin (AZM) is categorized as a macrolide. Because of its immunomodulatory, anti-inflammatory, and antibacterial properties, it offers numerous advantages. Azithromycin's special ability leads to its classification as a macrolide antibiotic. AZM is actively absorbed by a range of cells, including fibroblasts and white blood cells, due to its dual-base structure. This antibiotic drug functions in vitro against a variety of beta-lactam-resistant bacteria (such as Legionella and Chlamydia spp.) as well as numerous pyogenic bacteria (such as Neisseria gonorrhoeae [N.gonorrhoeae] and Moraxella catarrhalis [M. Catarrhalis]). Patients with various inflammatory illnesses of the respiratory tract can benefit from AZM because of its immunomodulatory, anti-inflammatory, and antibacterial modulatory properties. AZM has been used in clinical trials to prevent bacterial infections in patients with COVID-19 and has demonstrated effectiveness in these patients as well. It has been reported that hydroxychloroquine (HCQ) and azomiczine (AZM) together can reduce the SARS-CoV-2 virus load. Additionally, AZM has the ability to modify immune system characteristics, such as lowering cytokine production, preserving the integrity of epithelial cells, and averting lung fibrosis. The course of treatment with AZM is not very long (Heidary *et al.*, 2022).

2.7.3 Zinc

Both the innate and acquired immune responses against viruses are mediated by zinc. First and foremost, zinc's antioxidant and anti-inflammatory properties make it crucial for the mucosal epithelium's barrier function. Additionally, tight junction proteins—which are critical for preserving the integrity of the mucosal membrane—are regulated by it. Viral inflammation is exacerbated by loss of tight junction cohesion and a decrease in mucosal integrity. Acute respiratory distress syndrome is the result of these deteriorations, which cause alveolar oedema as a result of water and high-weight protein leaks. Zinc controls the maturation, differentiation, proliferation, and functionality of leucocytes, including lymphocytes. Innate and adaptive immune cells' intracellular signaling pathways are controlled by zinc ions. Zinc functions in two ways: either directly by reversibly binding to regulatory proteins or indirectly by influencing enzymes that are engaged in signaling pathways, such phosphates. The availability of zinc affects the production of cytokines and reactive oxygen species (ROS). Both the creation of neutrophil extracellular traps, which are produced by granulocytes to neutralize infections, and the intracellular death of pathogens depend on the generation of ROS. It has been shown that administering zinc in vitro increases leukocyte production of interferon α (IFNα), hence augmenting its antiviral efficacy. Zinc is involved in many immunological processes, hence a zinc deficit causes: immunological cell activity reduction, such as decreased phagocytosis, a reduction in essential neutrophil functions, diminished capacity of natural killer cells, a decrease in the quantity and activity of lymphocytes, diminished production of antibodies,

unbalanced cytokine secretion from T helper cells along with a reduction in IFNγ production (Immunostimulatory cytokines having antiviral properties are known as IFNs) and enhanced thymic atrophy and the ensuing infection risk (Mossink, 2020).

However, too much zinc can potentially compromise immune response by preventing T- and B-lymphocytes from functioning, decreasing macrophages' ability to destroy intracellular pathogens, or creating an overabundance of regulatory T-cells. This indicates that proper immune function depends on a balanced zinc homeostasis. Moreover, attaining a sufficient antiviral immune response necessitates precise regulation of the inflammatory response. Zinc plays a vital role in preventing over-reactions to inflammation by enhancing the regulation of nuclear factor-κB (NF-κB), a transcriptional factor that reduces the generation of pro-inflammatory cytokines. Zinc signals have the ability to raise intracellular concentrations of zinc finger protein A20, which in turn downregulates proinflammatory proteins by preventing NF-κB activation through a negative feedback loop. Zinc may also control the overabundance of inflammatory responses by altering the actions of regulatory T cells (Mossink, 2020).

Zinc has also demonstrated direct antiviral effect against a broad range of viruses, strengthening antiviral immunity. A zinc deficit increases the risk of contracting viral infections by impairing immune function and lowering zinc availability for direct antiviral action. As a result, populations with low zinc levels are more susceptible to viral illnesses such as hepatitis C and HIV as well as pneumonia. According to certain

research, zinc may also enhance the body's ability to fight off Streptococcus pneumoniae, a bacterium that can cause coinfection with viral pneumonia. Furthermore, zinc's anti-inflammatory properties help to prevent sepsis and limit tissue damage in the event of pneumonia. As our understanding of zinc's function in viral immunity has grown, clinical research has demonstrated the clear therapeutic benefit of zinc supplementation in the treatment of viral illnesses such as herpes simplex and the common cold. Because zinc has been clinically shown to be effective in reducing diarrhea, the World Health Organization currently recommends it as the first-line treatment for acute gastroenteritis in children, along with oral rehydration solution. How much zinc is also involved in COVID-19 antiviral immunity is the question (Mossink, 2020).

Reduced zinc availability appears to be associated with immunosenescence, the term used to describe the deterioration of immunological functioning with age. Mild zinc deficiency, which is frequently associated with aging, causes the adaptive immune system to become dysregulated, which lowers the particular immune response (B-cell activity, immunoglobulin synthesis), and increases the proinflammatory cytokine release, or "inflammaging." Zinc is continuously sequestered within cells during chronic inflammation, and zinc attached to intracellular proteins (metallothioneins) is not released as readily. Consequently, there is a decrease in intracellular zinc ion bioavailability, which is essential for healthy immunological activity. Supplementing with zinc somewhat reverses these changes. Additionally, innate immunity may be impacted by the inflammation that is frequently associated with aging. The innate

immune system plays a major role in generating a sufficient immune response, particularly in the early stages of newly acquired viral infections. Zinc in particular is essential to its effective operation. Similar to SARS and Middle East respiratory disease, COVID-19 is characterized by a high degree of inflammation and strong indications of downregulation of the innate immune response. This could help to explain why young children benefit from a very robust innate immune response, whereas the elderly are more susceptible to COVID-19s (Mossink, 2020).

2.7.4 Selinum

There is a lot of interest in the biochemistry and pharmacology of selenium right now, especially in terms of public health. Long recognized as an antioxidant in food, selenium is now understood to be a necessary part of the active sites of several enzymes, such as thioredoxin reductase and glutathione peroxidase, which catalyze reactions vital to shielding cellular constituents from oxidative and free radical damage. Numerous disorders, including as cancer, heart disease, osteoarthritis, and AIDS, have been linked to low plasma concentrations of selenium. As a result, numerous extensive clinical trials using selenium supplementation are currently in progress. There is growing evidence that selenium supplementation has an antitumorigenic effect on cancer, at least in part because it increases the creation of certain metabolites containing selenium rather than just maximizing the expression of selenium-containing enzymes. Consequently, several innovative pharmacological treatments based on selenium or focusing on particular facets of selenium metabolism are currently in research. These include organoselenium chemicals that lessen oxidative tissue damage and

edema, as well as oral-active antihypertensive, antiviral, anticancer, immunosuppressive, and antibacterial medications. It is expected that new selenium-based pharmacological compounds with therapeutic promise for a range of human disorders will be developed in the upcoming years as our understanding of the fundamental biology and biochemistry of selenium grows (May, 2002).

2.7.5 Lopinavir/Ritonavir – protease inhibitor

The proteases encoded by most viruses play a crucial role in the viral life cycle. The protease inhibitors (PIs) bind competitively to the substrate site of the viral protease. This enzyme is responsible for the post translational proteolysis of a polyprotein precursor and the release of functional viral proteins, allowing them to function correctly and individually in replication/transcription and maturation. Inhibition results in the production of immature virus particles. Coronavirus proteases, of which there are two in SARS-CoV (a papain-like cysteine proteinase (PLpro, nsp3) and a 3C-like proteinase (3CLpro or Mpro, nsp5)) and three in several other coronaviruses, cleave the ORF-1 polypeptide as it is translated, enabling the formation of the viral replication complex. The substrate-binding pockets are highly conserved among CoV 3CLpro, suggesting the possibility for a wide-spectrum inhibitor design targeting this region in the 3CLpro of all CoVs. It is postulated that the 3CLpro-inhibiting activity of lopinavir/ritonavir contributes at least partially to its anti-CoV effects. In silico binding studies of the drugs using the identified crystal structure of Mpro and employing the Hex program to conduct the docking of the ligands to the SARS-CoV main proteinase revealed that lopinavir and ritonavir could basically bind to the active

site of SARS main proteinase, but the efficacy of lopinavir/ritonavir was predicted to be poor. According to the latest report of the structure of 3CLpro from SARS-CoV2 (PDB code 6LU7) and the available structure of 3CLpro from SARS-CoV (PDB code 1UK4), the two main proteases differ by only 12 amino acids. Comparing ligand binding free energies for the main proteases has confirmed that good binders for SARS-CoV are, in general, also good binders for SARS-CoV2 3CLpro (Uzunova *et al.*, 2020).

Protease inhibitors, a class of medications well known for their effectiveness against HIV, effectively cure human immunodeficiency virus infection by blocking the last stage of virion assembly. When treating HIV infection, lopinavir with ritonavir is frequently used as a boosted protease inhibitor combination. In order to obtain medication concentrations high enough to limit viral replication, lopinavir must be coadministered with ritonavir due to its low oral bioavailability and substantial hydrolysis by the CYP3A4 isoenzyme (Uzunova *et al.*, 2020).

2.7.6 Ivermectin

In 1981, ivermectin, a dihydro derivative of avermectin (AVM), was brought to the animal health domains of veterinary medicine, agriculture, and aquaculture. Ivermectin was first prescribed for the treatment of the parasite disease onchocerciasis in 1987, but it was quickly accepted as a human medication. Ivermectin has since had a substantial impact on human health and welfare and has been utilized to treat a variety of different illnesses in people. Numerous published research have looked into the potential function of ivermectin in cancer within the last ten years. In order to define the

present state of advancement in the characterization of ivermectin, we have compiled the published papers into one review. Ivermectin induces immunogenic cell death (ICD) in cancer cell lines by modulating several pathways, such as the WNT-T cell factor (TCF), Hippo, and Akt/mTOR pathways. It also causes caspase-dependent apoptosis and PAK1-mediated cytostatic autophagy. Ivermectin is an RNA helicase, a small-molecule mimic of the surface-induced dissociation (SID) peptide, an activator of chloride channel receptors, an inducer of mitochondrial dysfunction and oxidative stress, and it can impact the growth and proliferation of cancer cells. Ivermectin also targets angiogenesis, has strong anti-mitotic activity, increases the multidrug resistance protein (MDR), and inhibits cancer stem-like cells (CSCs). Ivermectin exhibits its antitumor effects, as demonstrated by several research, and may therefore help cancer patients following adequate clinical trials (Liu *et al.*, 2020).

Although IVM has been shown to be effective in treating a wide range of parasite illnesses, its exact mechanism of action is unknown. IVM operates through ligand-gated chloride channels, particularly those controlled by glutamate, and impacts worm movement, eating, and reproduction at nanomolar doses. Since vertebrates lack glutamate-gated chloride channels (GluCls), it is believed that this contributes to the wide safety margin of IVM. IVM can, however, interact with a broader variety of ligand-gated channels at micromolar concentrations, such as GABA, glycine, histamine, and nicotinic acetylcholine receptors, which are present in both invertebrates and vertebrates. The effect of IVM on worm motion and eating is likely related to binding to GluCls at nematode motor neuron commissures, lateral and sublateral nerve cords,

and pharyngeal neurons, where GluCls is expressed. Five subunits make up functional GluCls, whereas native GluCls have a variety of subunit kinds. Six genes in the free-living worm Caenorhabditis elegans encode GluCl subunits; the primary target of IVM is glc-1. Even in closely related species, the GluCl family in parasitic worms appears to be extremely diverse. Haemonchus contortus, the gastrointestinal parasite of sheep, and Necator americanus and Anklesoma ceylanicum, the hookworms that infect humans, belong to the same evolutionary group as C. elegans, but none of them have glc-1 orthologues. Though different subunit combinations can produce functional GluCl channels, variations in the distribution and composition of GluCl channels may also play a role in the susceptibility of different nematode species to IVM. Notably, A. ceylanicum has been shown to be 40–300 times more susceptible to IVM than N. americanus in both in vitro and in vivo studies. These differences may also be due to differential sensitivity of the other ligand-channel types mentioned above. It has long been known that IVM inhibits the formation of microfilariae in utero, a fact that is well characterized by studies on filarial worms. IVM also interferes with nematode fertility. Since then, transcriptome analysis has revealed alterations in gene expression after female Briggia malayi was exposed to 100 nM–1 μM IVM in vitro, with transcripts that were differentially expressed being especially enriched for those related to female reproductive. Since there had not yet been any reports of GluCls in the nematode reproductive system, it was believed that IVM had an indirect impact on fecundity. Nevertheless, a GluCl component, avr-14, was identified by genome research of B. malayi, and this transcript was identified as being located in the reproductive system

of adult Brugia through the use of certain RNA probes.The highest levels of avr-14 expression were seen in microfilariae embryonic stages among other places, the female worm's uterine wall and, to a lesser degree, the male reproductive system; this finding could be useful in determining the mechanism underlying IVM-induced sterility. As observed in filarial nematodes, a parasite's sensitivity to IVM can change throughout its life cycle, and there is mounting evidence that the parasite's activity is influenced by interactions with the host immune system. GluCl in B. malayi microfilariae was localized exclusively to the tissue surrounding the excretory–secretory (ES) apparatus by means of an antibody against a peptide derived from AVR-14-A. It was suggested that IVM would lessen the amount of proteins released from the ES vesicle, potentially modifying host immune responses in vivo. This theory is in line with research on D. immitis microfilariae, which showed that peripheral blood mononuclear cells and neutrophils bound to IVM more readily when exposed in vitro. Furthermore, the in vitro effects of IVM for both D. immitis and O. volvulus microfilariae needed far greater doses than in vivo, suggesting a role for host immune function in the action of IVM (Laing *et al.*, *2*017).

2.8 What is an Adverse Drug Reaction?

A response to a medicine that is "noxious, unintended, and occurs at doses normally used in man" is the most often used definition of an adverse drug reaction (ADR). This definition, which has remained mostly unaltered, originated from a 1972 World Health Organization (WHO) report on International Drug Monitoring. On the other

hand, there have been significant changes to the classification schemes and terminology for allergy and anaphylaxis (Patton and Borshoff, 2018).

2.8.0 Types of Adverse Drug Reaction

Nowadays, there are two widely accepted classification schemes for adverse medication reactions. The most well-known initial classifications, type A or augmented (dose-dependent and predictable) and type B or weird (dose-independent and unpredictable), are frequently used to describe reactions that take place in critical care or anesthesia. Since then, types C–F have been introduced. Aronson and Ferner (2003) noted that certain adverse medication reactions did not always fall neatly into a single class and suggested a dosage-related, time-related, and patient-susceptibility-based system (DoTS). Still, the original type A and type B classification remains widely applicable, easy to use, and recognizable to doctors for many medication reactions found in anesthetics and critical care. Therefore, it continues to be utilized in the Medicines and Healthcare Products Regulatory Agency's (MHRA) ADR collection procedure. **(**Patton and Borshoff, 2018).

2.8.1 Hypersensitivity Reactions: Immediate Reactions

Antibiotics β-Lactam The conventional paradigm of reactions mediated by specific immunological mechanisms, including IgE antibodies, is still thought to apply to hypersensitivity reactions caused by β-lactam antibiotics (BLs). These antibiotics bind covalently to high-molecular-weight proteins, which the immune system may subsequently digest and detect. The exact mechanism by which this happens is still un-

known. Due to particular immunological pathways, BLs remain the most frequent cause of HDRs (Doña *et al.*, 2014).

Quinolones IgE and T lymphocytes have the ability to trigger hypersensitivity reactions in response to fluoroquinolones (FQs). Over 70% of instances with IgE-mediated reactions are severe; anaphylaxis and anaphylactic shock are the most prevalent clinical symptoms. IgE-mediated reactions are more common. Less frequently documented T cell-dependent reactions include Stevens-Johnson syndrome (SJS), MPE, acute generalized exanthematic pustulosis, fixed drug eruptions, and toxic epidermal necrolysis (TEN). Phototoxicity accounts for a significant fraction of T-cell reactions. Over the past ten years, FQs have become more common than any other non-BL antibiotic in HDRs, as seen by the rise in HDR prevalence caused by these antibiotics. The most often used FQ is levofloxacin, followed by ciprofloxacin and oxyfloxacin. The most often used FQ is levofloxacin, followed by ciprofloxacin and oxyfloxacin. Additionally, compared to other FQs, moxifloxacin causes more severe responses (Doña *et al.*, 2014).

2.8.2 Non-immediate Reactions

Antibiotics Non-immediate reactions (NIRs) consist of a spectrum of entities that usually occur within 24 to 48 hours of drug exposure, although the time can be as short as 1 to 2 hours following an exposure period of 48 hours or more. While benign diseases like MPE and urticaria are the most common, more serious responses including drug rash with eosinophilia and systemic symptoms (DRESS) and TEN can also happen. Variations in the underlying pathophysiological pathways account for the various clin-

ical presentations, and distinct T-cell populations are implicated in each of these entities. Due to the challenges of gathering a trustworthy clinical history, the significance of recognizing concomitant conditions such viral infections, and the low sensitivity of skin and in vitro tests, diagnosis is frequently complicated. The most effective—and frequently the only—method to establish a link between a drug's use and a negative immune response is drug provocation testing. Nevertheless, in certain cases—such as acute generalized exanthematous pustulosis, SJS, TEN, DRESS/drug-induced hypersensitivity syndrome, systemic vasculitis, specific organ manifestations (blood-cytopenia, hepatitis, nephritis, pneumonitis), generalized bullous fixed drug eruptions, and drug-induced autoimmune diseases—it is not generally advised and is even advised against (Doña *et al.*, 2014).

2.8.3 Radio Contrast Media

Over the past ten years, there has been a rise in NIRs to radio contrast media (RCM) concurrent with an increase in the compounds' use. Due to the nonspecific release of vasoactive mediators, the high osmolarity of ionic RCM was previously linked to a high prevalence of rapid responses. The frequency of acute reactions to RCM declined after nonionic low-osmolarity RCM was introduced in the 1970s. On the other hand, NIRs have become more frequent to the point where they now outweigh immediate reactions. This could be explained by T cells' improved understanding of the molecular nature of nonionic RCM (Doña *et al.,* 2014).

2.8.4 Neuromuscular Blocking Agents

In the past few decades, perioperative allergic responses have received more and more attention. These can be divided into two categories: IgE-dependent allergic reactions and reactions arising from direct nonspecific mast cell and basophil activation. This classification depends on the underlying mechanism. In contrast to IgE-mediated reactions, direct histamine release reactions are typically milder. Unknown is the actual incidence of perioperative responses. The figures are not consistent, which is likely due to variations in reporting systems and clinical practices between nations. Anaphylaxis is thought to occur in 1 in 10,000 to 1 in 20,000 occurrences of all immune- and nonimmune-mediated acute hypersensitivity reactions, which is predicted to occur in 1 in 5000 to 13 000 anesthetic procedures. On the other hand, most people believe that immediate-type hypersensitivity reactions are significantly underreported (Doña *et al.*, 2014).

2.8.5 Hypersensitivity Reactions by Other Mechanisms

Cross-Intolerance: While many medications have the ability to trigger the release of histamine or other mediators through non-specific immunological mechanisms, NSAIDs have received increased attention in recent years. Due to their widespread use, this class of medications is now linked to a number of negative side effects, including hypersensitivity reactions [93,94]. Actually, the most prevalent class of medications used in HDRs these days is NSAIDs. Reactions have been divided into two groups. The first is called cross-intolerance (CI), and its suggested mechanism involves the release of sulphidopeptide leukotrienes and histamine together with the in-

hibition of the COX enzyme. Multiple NSAIDs with unrelated chemicals may be the cause of this. Patients in the second type, known as selective responses (SRs), respond well to a single NSAID and have good tolerance to additional NSAIDs that are chemically unrelated. According to our experience, the first category of reactions makes up more than 75% of instances and is the most prevalent. Five subgroups can be distinguished from these two basic groupings. This is the most recent classification of NSAIDs that has been suggested by the European Network of Drug Allergy interest group. More subclassification with additional traits has been suggested. CI to NSAIDs may have an impact on the pulmonary airways, the skin, or both. Early research on NSAID hypersensitivity reactions concentrated on conditions involving the respiratory system, such as nasal polyposis, rhinitis, and/or asthma. But in both the CI and SR groups, the most often damaged organ is the skin. Two cutaneous conditions have been identified: NSAID-induced urticaria/angioedema (NIUA), which is acute urticaria/angioedema in the absence of a history suggestive of chronic spontaneous urticaria, and NSAID-exacerbated cutaneous disease (NECD), which is the exacerbation of preexisting chronic spontaneous urticaria. While NIUA is more frequent, most investigations on CI with skin involvement have been conducted in patients with NECD. The natural progression of NIUA is a topic of controversy; some authors suggest that it can lead to NECD. In a recent study, 6% of a sizable cohort of NIUA patients followed for a 12-year period experienced persistent spontaneous urticaria, a rate comparable to that of the control group. Propionic acid derivatives have been used more frequently in recent years, which has led to an increase in reports of side effects, such as renal

failure, acute myocardial infarction, heart failure, and hypersensitivity reactions. Pyrazones and acetylsalicylic acid (ASA) were the drugs most frequently involved in hypersensitivity reactions to NSAIDs between 1980 and 1990; pyrazolones decreased between 1991 and 2000; and propionic acid derivatives were the most common between 2001 and 2010, followed by ASA in second place and pyrazolones in last place, according to an analysis of the drugs involved in NSAID-induced HDRs over the last 30 years. These modifications might be partially explained by how each NSAID's consumption habits have changed over time (Doña *et al.*, 2014).

2.8.6 Pharmacovigiliant reactions

The effects of a drug include pharmacodynamic effects and side effects. *Pharmacodynamic effects* are the characteristic drug effects that are of interest for treating diseases; examples include an ability to decrease serum uric acid levels or to suppress inflammation. Phase I and II human investigations, followed by Phase III clinical trials, are where pharmacodynamic effects are first reported in animal research. As long as the dosage is sufficient, they happen to everyone (Montastruc *et al.*, 2006).

2.8.7 Idiosyncratic adverse drug reactions

In addition to being a major cause of patient morbidity and death, idiosyncratic medication responses significantly raise the uncertainty surrounding new drug development. The epidermis, liver, and bone marrow are the main targets. Clinical features point to immune-mediated disorders (IDRs), and there is strong evidence that chemically reactive species are the cause of most IDRs, but not all of them. However, con-

ducting thorough mechanistic research is highly challenging, particularly in the lack of reliable animal models. The hapten and P-I models are among the models used to describe how medications or reactive metabolites interact with the MHC/T-cell receptor complex. More recently, it was discovered that abacavir can interact reversibly with MHC to change the endogenous peptides that are presented to T cells. Although the percentage of IDRs that have a strong HLA reliance is yet unknown, the identification of HLA molecules as substantial risk factors for some IDRs has also made a significant contribution to our understanding of these adverse reactions. Moreover, most individuals who have the HLA that increases the likelihood of an adverse drug reaction (IDR) will not experience an IDR when treated with that medicine, with the exception of abacavir. The patients who will develop an IDR are likely to be selected by reasons other than interindividual variations in T-cell receptors. A medication that may generate immunological-related side effects (IDRs) may elicit a dominant immune response, immune tolerance being one such delicate balance (Uetrecht and Naisbitt, 2013).

2.9 Toxicology Screening

Toxicology is a branch of science that deals with toxins and poisons and their effects and treatment. Toxicological screening is very important for the development of new drugs and for the extension of the therapeutic potential of existing molecules. According to the US Food and Drug Administration (FDA) (21CFR Part 314), it is critical to screen novel compounds for pharmacological activity and potential animal toxicity. In the twenty-first century, the harmful impacts of foods, medicines,

chemicals, and other substances have become increasingly important (Parasuraman, 2011).

2.9.0 Acute toxicity Testing

Acute toxicity testing is used to find out how a single dose affects a certain type of animal. Acute toxicity testing should generally be performed on two distinct animal species (a rodent and a nonrodent). Acute toxicological testing involves the administration of the investigational substance at various dose levels, with the effect being monitored for a period of 14 days. The investigational product is responsible for all recorded mortalities that occur during the trial time. Additionally, alterations in the morphological, biochemical, pathological, and histological aspects of the deceased animals are examined. The 50% lethal dose (LD50) of the experimental product can be ascertained by acute toxicity testing. Previously, the LD50 was employed as a measure of acute toxicity. A high mortality ratio and a large number of animals are used in the LD50 estimation process. Owing to these drawbacks, three modified techniques were created: the up-and-down (UDP) method, the acute toxic category (ATC) approach, and the fixed dosage process (FDP). Rather than the lethal dose, the nonlethal toxicity is evaluated using the FDP. The experimental animal is monitored for a predetermined amount of time while the investigational substance is given to it at fixed dosage levels of 5, 50, 500, and 2000 mg/kg. Three animals of the same sex are utilized in each step of the ATC technique, which is a sequential process.Four predetermined starting doses may be utilized in the ATC screening procedure, and the Globally Harmonized Classification system should be used to select the test dose. The

staircase design is another name for the UDP testing methodology. Various regulatory organizations endorse this methodology for toxicological testing since it minimizes the use of vertebrate animals in research. In the UDP screening approach, individual animals are dosed one after the other at 48-hour intervals. It is best to use female rats for evaluating UDPs.An animal is given a dose that is lower than the best-estimate LD50 value, and it is then monitored for 48 hours. If the animal lives, the study is carried out again using double the initial dose; if it dies, testing is done at a lesser dose on an additional animal of the same sex. The dose range for UDP testing is up to 2000 mg/kg. Different testing protocols are performed for dosages between 2000 and 5000 mg/kg. A single dose acute toxicity assessment method for pharmaceuticals was proposed by the Center for Drug Evaluation and Research (CDER) in 1996. This method uses a fixed safe dose that shouldn't result in adverse effects or endanger an animal's life. A minimum of two mammalian species, including a nonrodent species, must be used in the experiment, and the animals must be monitored for a period of 14 days **(**Parasuraman, 2011).

2.9.1 Subchronic oral toxicity testing (repeated dose 90-day oral toxicity testing)

The subchronic toxicity of a chemical is studied in both rodents and non-rodents. Oral administration of the test material is carried out for ninety days during which behavioral, biochemical, and cardiovascular parameter changes, weekly fluctuations in body weight, and monthly alterations are noted. The experimental animals are killed at the conclusion of the study. All of the tissues undergo histological examinations, and significant pathological alterations are noted. The animals should

have minimal individual variation, with a weight variation range of ±20% permitted. The protocol for the study may include a satellite group that consists of a control group and a high-dose group **(**Parasuraman, 2011).

2.9.2 Chronic oral toxicity testing

Studies on chronic toxicity involve at least one rodent species and one nonrodent species. The animals receive the test substance for a period of more than 90 days, and they are routinely examined. A chronic toxicological study draws conclusions about an experimental substance's long-term effects on animals, which can then be extrapolated to the substance's safety in humans. For novel pharmacological entities, the chronic oral toxicity data is crucial. The range of acceptable weight variation is ±20%, and there should be minimal individual variance among the animals. The study procedure might involve a satellite group. There is a control group and a high-dose group in this group. The animals are monitored for normal physiological functioning, behavioral changes, and changes in biochemical indicators during the study period. After the investigation is over, tissues are taken from every portion of the animal and examined histologically **(**Parasuraman, 2011).

2.9.3 Carcinogenicity Testing

Both rodents and nonrodent animal species may be used in carcinogenicity testing. The tests are carried out over the greater portion of an animal's lifespan. During and after exposure to test substances, the experimental animals are observed for signs of toxicity and development of tumors. If these are not found, a test may be terminated

after 18 months in the case of mice and hamsters and after 24 months with rats. If the animals are healthy, hematological analysis is performed after the 12 months and the 18 months, respectively, and the study is terminated. The animals are sacrificed, and gross pathological changes are noted and histopathological studies are carried out on all the tissues **(**Parasuraman, 2011).

2.9.4 One-Generation Reproduction Toxicity Testing

Both male and female animals are given the test chemical. For male animals, the duration of administration is one whole spermatogenic cycle; for female animals, it is two entire estrous cycles. For the toxicity testing of one-generation reproduction, rats are the favored model. The animals are permitted to mate when the prescribed amount of time has passed after the drugs have been administered. The test substance is given to the female animals when they are feeding and pregnant. A collection of male animal sperm is made, and its motility and morphology are examined. The animals are watched for indications of toxicity during the study time. The number of children, their sexes, and the parturition are all documented. Throughout the first four days, the number of dead and living puppies is recorded, and the live pups are weighed every morning and evening. The animals and puppies are slaughtered and put through a histological investigation when the study comes to an end **(**Parasuraman, 2011).

2.9.5 Two-Generation Reproduction Toxicity Studies

The test material is fed to both male and female rodents. One full spermatogenic cycle for males and two full estrous cycles for females are covered by the administration

period. The animals mate (parental mating) following the administration period, and the female animals are then separated. Male animals provide their sperm, and their motility and morphology are examined. Pregnant female animals receive the test drug constantly, and their mortality and toxicological symptoms are periodically observed. Following parturition, the test medication is given to nursing rats, and the pups' (F1 generation) mortality is monitored. Two animals—one male and one female—are chosen from the F1 generation. For the F2 generation offsprings, the same process is performed. F1 kids are assessed for infertility if a pair does not become pregnant within the allotted time after reaching complete sexual maturity. It is necessary to perform necropsies and histology exams. The animals are slain at the conclusion of the study, and each one undergoes extensive pathological and histological testing **(**Parasuraman, 2011).

2.9.6 Genetic Toxicity Testing

To find gene mutations, chromosome changes, and modifications in the DNA sequencing, genetic toxicity tests are utilized. Usually, a variety of species are used for these studies, including complete animals, plants, microorganisms, and mammalian cells. Rats are the preferred animal in the entire animal model. The rodent chromosome assay, dominant lethal assay, mouse-specific locus test, micronucleus test, heritable translocation assay, and sister chromatid exchange assay are used to evaluate genetic toxicity **(**Parasuraman, 2011).

Numerous studies have observed a decrease in total cholesterol, LDL-c, HDL-c, and apolipoprotein B and A-I levels in patients with COVID-19 infections, similar to what is observed with other infections. In most studies the decrease in LDL-c and/or HDL-c was more profound the greater the severity of the illness. LDL-c and HDL-c levels were inversely correlated with C-reactive protein (CRP) levels i.e., the lower the LDL-c or HDL-c level the higher the CRP levels. Patients with low HDL-c and/or LDL-c levels at admission to the hospital were at an increased risk of developing severe disease compared to patients with high levels. With recovery from COVID-19 infections the serum lipid levels return towards levels present prior to infection. In patients that failed to survive, total cholesterol, LDL-c, and HDL-c levels were lower at admission to the hospital and continued to decline during the hospitalization. In patients with COVID-19 infections the serum triglyceride levels were variable. Lipoprotein (a) levels increase during COVID-19 infections. Several studies using the UK Biobank and other databases have shown that low HDL-c and apolipoprotein A-I levels measured many years prior to COVID-19 infections were associated with an increased risk of COVID-19 infections and death from infection while LDL-c, apolipoprotein B, lipoprotein (a), and triglyceride levels were not consistently found to be significantly associated with an increased risk. A 10 mg/dl increase in HDL-c or apolipoprotein A1 levels was associated with ~10% reduced risk of COVID-19 infection. It should be noted that these observations are subject to the caveats of confounding variables and reverse causation effecting the results. Several studies have found that homozygosity for apolipoprotein E4/4 is associated with a 2-3- fold increased risk of COVID-19 in-

fections and this increase was not due to dementia or Alzheimer's disease. During the COVID-19 pandemic, diet, exercise, and lipid lowering therapy should be continued. For those who become symptomatic, lipid lowering therapy, if feasible, should also be continued throughout the duration of the illness. Individuals who are naïve to treatment but for whom lipid lowering therapy is indicated should be started on treatment. Whether lipid lowering drugs have beneficial effects when given prior to or during COVID-19 infections is uncertain but randomized controlled studies are in progress. In patients with severe symptoms of COVID-19 who are too ill to take oral medications, lipid lowering medications may be temporarily suspended. Medications should be re-started when the patient has recovered and able to take oral medications. One needs to be aware that certain drugs that are used to treat COVID-19 infections may interact with lipid lowering drugs. Remdesivir and Paxlovid (nirmatrelvir and ritonavir) are metabolized by the Cyp3A4 pathway and statins that are also metabolized by this pathway should be avoided (atorvastatin, simvastatin, and lovastatin). Because drug therapy for patients with COVID-19 infections is rapidly evolving one needs to be alert for potential drug interactions (Feingold, 2022).

HCQ is a 4-aminoquinoline marketed as Plaquenil®, and is used in the treatment for malaria and rheumatic and inflammatory diseases (rheumatoid arthritis and lupus erythematosus). HCQ has anti-inflammatory and analgesic actions, which may be of benefit during SARS-CoV-2 infection. Interestingly, recent studies hypothesizes that HCQ modifies endosomes pH and inactivates viruses during their infections. The cytochromes P450 2D6 and 3A4 (CYP2D6 and CYP3A4) participate in the N-

dealkylation of HCQ to the active metabolite, desethylhydroxychloroquine (DHCQ), as well as in the generation of the inactive metabolites, desethylchloroquine (DCQ), and bidesethylchloroquine. DHCQ is the main metabolite found in the blood and urine. Chloroquine has demonstrated antiviral effects, in particular on the viral replication of several coronaviruses, including SARS-CoV and MERS-CoV. Up to 100 µM of HCQ does not cause any harm when used to prevent SARS-CoV-2 infection of Vero6 cells in vitro. The effectiveness of HCQ in treating COVID-19, however, has been the subject of conflicting findings in recent research. The issue of compromised HCQ metabolism and pharmacokinetics under specific clinical circumstances comes up in this context, and it may be able to shed light on the variations in efficacy seen in patients and in vitro. Therefore, research on HCQ metabolism in diseases associated with COVID would be especially interesting (Ferron *et al.,* 2021).

2.10 Lipids

Triglycerides, cholesterol, and fatty acids are examples of lipids that are broken down and biosynthesised as part of lipid metabolism. Transport of lipids from the stomach to the liver, the site of most lipid changes, and between the liver and peripheral organs is facilitated by specialized lipoproteins (Schoeler and Caesar, 2019).

Lipids are organic substances that dissolve in organic solvents but not in water. Triglycerides, phospholipids, and steroids are among the esters of fatty acids that are rarely associated with alcohol or phosphate functional group molecules. They act as the body's energy stores, carry out a number of tasks like regulating body temperature, and are essential components of cell membranes and chemical messengers. The hu-

man body needs a variety of beneficial lipid fats to keep all of its components functioning properly. Maintaining blood lipid balance is critical to overall health. Fat deposits in artery walls are a result of abnormal blood lipid levels, and this leads to problems inside the blood arteries. Diabetes, alcoholism, renal disease, hypothyroidism, liver disease, and stress are among the factors that contribute to high lipid levels. Increased lipids readily attach themselves to the blood's circulating nerve walls, leading to an increase in fatty scale and a host of atherosclerosis conditions, including heart attacks and strokes (Natesan and Kim, 2021).

2.11 Cholesterol: Cholesterol metabolism has been studied for many decades. In mammals, the products of cholesterol metabolism are bile acids, and steroid hormones and their metabolites. While bile acids and steroid hormones are of undoubted importance, in recent years interest has shifted to intermediates in their biosynthesis and to a category of molecules known as oxysterols Oxysterols can be defined as oxidised forms of cholesterol or of its precursors. They are formed in the first steps of cholesterol metabolism, mostly by cytochrome P450 (CYP) enzymes. They can also be formed via nonenzymatic reactions both *invivo* and *exvivo*. Numerous oxysterols have biological activity as ligands for receptors such as glutamate, G protein-coupled receptors (GPCRs), and nuclear receptors. Reduced production of the antiviral compounds 25-HC and 26-HC may be a result of a major pathophysiological feature of COVID-19: lower cholesterol availability to cells. However, it may be anticipated that lower serum cholesterol might be helpful in protecting against COVID-19 given on

research demonstrating 25-HC is antiviral via limiting cholesterol availability to membranes (Wang *et al.*, 2021).

2.12 Triglycerides

Studies on epidemiology and genetics demonstrate that TRL and its byproducts have a significant role in ASCVD. The current guidelines recommend apoB and non-HDL-c as secondary objectives that reflect the contribution of TRL, in addition to LDL-c, the primary therapeutic target for lipid-lowering medication. Putting lifestyle modifications into practice is the first stage in treatment. Second, reducing LDL-c with statins is advised to lower the risk of cardiovascular disease; this is separate from the statin-associated lowering of TRL. In individuals with optimum cholesterol-lowering, prior trials utilizing fibrates, niacin, and CETP inhibitors did not yield definitive proof of a reduction in ASCVD. Nonetheless, current and future research highlights the significance of TRL for residual risk in statin-treated ASCVD patients. New and developing information, such as that on specific PPAR modulators like pemafibrate and omega-3 fatty acids (high-dose icosapent ethyl), may help identify people who will benefit from TRL reduction. Novel therapeutic targets that are undergoing clinical trials have emerged as a result of our growing understanding of the molecular processes underlying TRL. These include intestinal diacylglycerol acyltransferase inhibitors (pradigastat), inhibitors of apo C-III antisense (volanesorsen), inhibitors of angiopoietin-like protein 3 (evinacumab; ASO IONIS-ANGPTL3-LRx), and therapies aimed at apo C-II, A-V, and angiopoietin-like protein 4.To sum up, TRL have become significant indicators of residual risk and targets for treatment. Numerous current studies will docu-

ment the effectiveness of cutting-edge pharmacologic techniques to reduce serum TG levels. In addition to reducing LDL-C, we hypothesize that addressing TRL in particular patient populations may eventually become a crucial component of lipid-directed treatment (Laufs *et al.,* 2020).

2.13 Phospholipids

Phospholipids are molecules in which hydrophilic head group and hydrophobic acyl chains are linked to the alcohol. The variation in head groups, aliphatic chains and alcohols leads to the existence of a wide variety of phospholipids. In addition, the different sources of phospholipids also enhance the species of phospholipids. Various phospholipids, such as soybean phosphatidylcholine, egg phosphatidylcholine, or synthetic phosphatidylcholine, as well as hydrogenated phosphatidylcholine, are commonly used in different types of formulations. Phospholipids become intriguing as they can offer various options. However, the species diversity of phospholipids make how to select an appropriate phospholipid to achieve the therapeutic purpose become a crucial problem in the design of DDS, so we summarized the structures, main sources, properties of phospholipids which can give a guideline in the design of DDS. In addition, we set liposomes, intravenous lipid emulsions, PC/bile salt mixed micelles, phospholipid micelles, drug-phospholipid complexes, cochleates as examples to introduce the main applications associated with phospholipids and further explain how to make a choice among the phospholipids in drug delivery. Phospholipids are lipids containing phosphorus, a polar potion and non-polar potion in their structures. According to the alcohols contained in the phospholipids, they can be di-

vided into glycerophospholipids and sphingomyelins. Phospholipids can be classified as natural or synthetic phospholipids based on the sources (Li *et al.,* 2015).

2.14 Esters

Viscous, water-immiscible liquids, containing only carbon, hydrogen, and oxygen (Boyde, 2020).

2.15 Lipoproteins/Glycoproteins

Typically, glycocans are present on cells as glycolipids or glycoproteins, where they are covalently bonded to lipids or proteins, respectively (Shajahan et al., 2017). Rather than analyzing the glycoprotein as a whole, most glycoprotein analyses are top-down, which involves cleaving the glycoprotein into smaller pieces (Shajahan et *al.*, 2017).

- LDL is directly atherogenic because its small size allows it to infiltrate into the walls of inflamed vascular endothelium (Each 1mg/dL increase in LDL increases the risk of a coronary artery event by 1%-1.5%). Normally LDL is not irritating to the tissues but when it lingers too long in the oxygen rich subendothelium, its lipids become peroxidized whereupon the LDL particle is attracted to the scavenger receptor of a nearby tissue macrophage & is ingested.(Hastings, 2005).

- High Density Lipoprotein (HDL) conveys cholesterol back to the liver. HDL contains Apo-A lipoproteins and is antiatherogenic (every 1mg/dL increase in plasma HDL confers a 1%-1.5% decrease in the probability of a coronary artery event) (Hastings, 2005).

- VLDL & IDL, if present in excessive quantities may be indirectly atherogenic. The reason is that both have about 20 times stronger an affinity for the LDL receptor than does LDL (That's because both VLDL & IDL contain apo-E, which confers this higher affinity, whereas LDL does not) (Hastings, 2005).

2.16 Chylomicrons

Chylomicrons, which are lipoproteins rich in triglycerides, are formed from dietary lipids by the small intestine and then circulated to other tissues. The processes of chylomicron synthesis and secretion, as well as intestinal lipid absorption and mobilization, are strictly controlled. In common metabolic illnesses including insulin-resistant states and type 2 diabetes, elevated chylomicron production rate is linked to dyslipidemia and may also raise the risk of atherosclerosis in these circumstances. Comprehensive knowledge of the regulation of chylomicron formation may lead to the discovery of pharmaceuticals with potential therapeutic benefits in the avoidance of dyslipidemia and atherosclerosis. A number of variables, including body weight, nutrition, hormones, genetic variations, nutraceuticals, drugs, and newer therapies like bariatric surgery, might affect chylomicron secretion (Dash *et al.*, 2015).

2.17 Absorption, Transport & Intermediate Metabolism of Fats

Absorption & Transport: In contrast to glucose, fats (triglycerides & cholesterol) do not pass directly through the wall of the gut & into the bloodstream. Instead, they are loaded & enfolded in the mucosa into the core of a type of lipoprotein structure called chylomicrons. Chylomicrons then carry the load of triglycerides & cholesterol toward

the liver via the thoracic duct. On the way from the gut to the liver, HDL particles transfer apolipoproteins C II & C III to the chylomicrons. Lipoprotein lipase (LPL), an enzyme found in the endothelial cells of adipocytes and muscle cells, is activated by Apo C II and inhibited by Apo C III. LPL then hydrolyzes the triglycerides delivered by the chylomicrons, liberating fatty acids that are subsequently directly absorbed by adipocytes and muscle cells. Apo-C II and Apo-C III are then recycled back into HDL. After that, the "chylomicron remnant" binds to the hepatic LDL receptor or LDL-receptor-related protein (LPR) through the transfer of apolipoprotein-E (apo-E) from the hepatocyte. In order to create bile acids, steroid synthesis, or structural membranes, the chylomicron remnant discharges its cholesterol load into a freshly formed extremely low density lipoprotein particle. This lipoprotein particle is then recycled.VLDL changes into intermediate-density lipoprotein (IDL) as its triglyceride content is offloaded downstream. It then transforms into an LDL particle by transferring its apo-E to. LDL makes over 70% of the lipids in circulation. Triglycerides and cholesterol are transported where they are needed by lipoproteins, which function similarly to envelopes. In addition to transporting triglycerides to muscle cells and adipocytes, chylomicrons also transfer cholesterol from the stomach to the liver. The body uses low-density lipoprotein (LDL), intermediate-density lipoprotein (IDL), and very-low-density lipoprotein (VLDL) to transport triglycerides and cholesterol from the liver to the other organs. Apo-B lipoproteins are present in LDL, IDL, and VLDL. Because of this, the majority of commonly employed markers are not as good indicators of atherogeic potential as the plasma apo-B content (Hastings, 2005).

2.18 Very Low Density Lipoproteins (VLDL) AND Intermediate Lipoproteins (IDL)

VLDL & IDL, if present in excessive quantities may be indirectly atherogenic. The reason is that both have about 20 times stronger an affinity for the LDL receptor than does LDL (That's because both VLDL & IDL contain apo-E, which confers this higher affinity, whereas LDL does not). After formation, the larger, more lipid-laden VLDL particles remain in the liver sinusoids attached via apo-E to the hepatic LDL receptor. Smaller VLDL particles are released & progressively degraded to IDL & LDL. The protein called cholesterol-ester-transfer-protein (CETP), which circulates with HDL, typically catalyzes the conversion of HDL cholesterol esters into chylomicrons or VLDL in exchange for triglycerides. When LDL is overloaded with extra triglycerides, as is often the situation in diabetics, it may also transfer cholesterol esters to LDL and does so with considerable abandon. This produces dense, tiny LDL particles that are extremely atherogenic because they more easily penetrate the endothelium (Hastings, 2005).

2.19 Low Density Lipoproteins (LDL)

LDL is directly atherogenic because its small size allows it to infiltrate into the walls of inflamed vascular endothelium (Each 1mg/dL increase in LDL increases the risk of a coronary artery event by 1%-1.5%). Normally LDL is not irritating to the tissues but when it lingers too long in the oxygen rich subendothelium, its lipids become peroxidized whereupon the LDL particle is attracted to the scavenger receptor of a nearby tissue macrophage & is ingested. The macrophage's scavenger receptor is always "turned on". It is unregulated so it ingests peroxidized cholesterol until the macro-

phage's cytoplasm becomes so engorged that it looks like a "foam cell" under the microscope & after stuffing its cytoplasm with even more cholesterol the macrophage dies. That's how "lipid streaks" are formed. They're present in most American children by age 11. LDL carries apolipoprotein B & uses apolipoprotein B to attach to the LDL receptor. Lipoprotein (a) is a variant form of LDL. It is simply an LDL particle that has an extra apolipoprotein (apolipoprotein [a]) attached to its apo-B component by a sulfhydryl link. This makes the lipoprotein (a) structurally similar to plasminogen. Normally, tPA converts plasminogen to plasmin & plasmin lyses blood clots. Lipoprotein (a) is similar enough to plasminogen, that it competes with its receptor, blocks its conversion to plasmin & interferes thrombolysis. Lipoprotein (a) is therefore thrombogenic & atherogenic & its plasma levels predict coronary artery risk. Niacin and estrogens reduce lipoprotein (a) levels (Hastings, 2005).

2.20 High Density Lipoproteins (HDL-c)

HDL, or high density lipoprotein, returns cholesterol to the liver. HDL contains Apo-A lipoproteins and is antiatherogenic (every 1mg/dL increase in plasma HDL confers a 1%-1.5% decrease in the probability of a coronary artery event). The structural apolipoprotein for HDL is called Apo-A I. It combines in the following ways with apo-A II & apo-A IV to generate the different forms of HDL: The apo-A lipoproteins are the only ones found in the developing HDL complex. Subsequently, the developing HDL particle obtains apo-C from lipoproteins rich in triglycerides, transforming into HDL3. The next step is for free cholesterol to enter the center of the HDL particle, where LCAT transforms it into cholesterol ester, increasing the particle's size and

buoyancy. This particle is now known as HDL2. After being transferred to chylomicrons or VLDL, HDL2 may then return the cholesterol load to the liver. This process is called “reverse cholesterol transport”. It accounts for the antiatherogenic properties of HDL (Hastings, 2005).

Apolipoproteins are like the address on an envelope that tell where the envelope is to be delivered. Apolipoprotein A is anti-atherogenic. There are 3 forms: apo-A I, apo-A II & apo-A IV. All are present on HDL & on chylomicrons. Apo- AI is the principle structural protein in HDL, interacting synergistically with apo-A II & apo-A IV. Apo-A-IV facilitates transfer of apolipoproteins between HDL & chylomicrons. An atherogenic apolipoprotein B exists. There are two types: apo-B 48, which is found on chylomicrons and is required for the production and excretion of chylomicrons in the stomach. The structural protein of VLDL, IDL, and LDL is called Apo-B 100. It is necessary for the liver's production of VLDL. It binds to the LDL receptor as well. One of the more accurate indicators of coronary artery risk is the plasma apo-B level. Apolipoprotein C is found on LDL, VLDL, IDL, and chylomicrons. Three forms exist: The liver's absorption of chylomicrons and VLDL remains is inhibited by apo-C I and apo-C III. Apo-C II makes LPL active. LPL is inhibited by Apo-C III. Apolipoprotein E can be found in VLDL, HDL, IDL, and chylomicrons. It has a 20-fold higher affinity than apo-B, the only ligand on LDL that binds to the LDL receptor. Additionally, apo-E ligands for LRP and a particular apo-E receptor on hepatocytes (Hastings, 2005).

2.21 Exogenous & Endogenous Factors of Lipid Metabolism

It is evident that all lipoproteins' functionality and metabolism can be altered by endogenous (such as genetic susceptibility, epigenetics, disease status) and exogenous (such as pharmacological treatments, nutrition, and lifestyle choices) variables. Taken together, these elements could play a role in the development and course of harmful long-term conditions such inflammation and oxidative stress. These circumstances are the primary cause of HDL's altered biological activity and role as well as the development of oxidized LDL (Poznyak et al., 2020). Therefore, researching modifiable factors that may affect lipoprotein metabolism and functionality may aid in the identification of potential intervention options to reduce the risk of acquiring illnesses (Cervellati *et al.,* 2022).

2.22 Disorders of Lipid Metabolism

Disorders of lipid metabolism are very common. They play an important role in the pathogenesis of atherosclerosis and can be effectively treated by lifestyle changes and drugs (Parhofer, 2016).

To the present day, familial hypercholesterolemia-characterized by pronounced coronary heart disease (CHD) at an early age and very high LDL cholesterol levels-remains the most cogent, convincing proof of the close causal connection between elevated LDL cholesterol and atherosclerosis. Many recent studies have shown, however, that familial hypercholesterolemia is just the extreme expression of this relationship. The link between LDL cholesterol and atherosclerosis extends over the whole

spectrum from genetically determined very low levels to extremely high concentrations (Parhofer, 2016).

Secondary lipid metabolism problems can be the result of several diseases. From a clinical standpoint, the most significant ones are hypertriglyceridemia or mixed hyperlipoproteinemia associated with diabetes mellitus; hypothyroidism associated with LDL hypercholesterolemia; kidney diseases associated with lipoprotein (a) elevation, mixed hyperlipoproteinemia, and hypercholestatic liver diseases associated with apparent elevation of LDL cholesterol. Lipid metabolism abnormalities are also noted in conjunction with other illnesses (e.g., lymphoma, Cushing syndrome, and porphyria). Treatment for lipid metabolism disorders that are secondary manifestations of underlying diseases should take precedence. Individuals with renal disease or diabetes mellitus frequently defy this rule because they exhibit features of both primary and secondary lipid metabolism disorders and are not able to achieve adequate management or eradication of the underlying illness (Parhofer, 2016).

Owing to its close association with the metabolic syndrome, mixed hyperlipoproteinemia, in which the concentrations of both LDL cholesterol and triglycerides are raised, is the most frequently occurring disorder of lipid metabolism in diabetics. Here too, the primary treatment goal is regulation of the LDL cholesterol level. To this end a statin is prescribed, perhaps in combination with ezetimibe. With regard to the hypertriglyceridemia, modification of the patient’s lifestyle is the key measure. If this combination of lifestyle modification and statin treatment does not achieve the target concentrations or at least normalize the triglyceride level, combined medicinal treat-

ment can be considered. In principle, a statin can be administered together with omega-3 fatty acids or fibrates, but both of these combinations have performed disappointingly in endpoint studies. Because these studies were poorly designed, however, no definitive conclusion can be drawn: each of these two groups of substances reduced the cardiovascular risk in monotherapy studies. In our center, therefore, after exhaustion of the LDL cholesterol-lowering options, patients with very high risk and a mixed lipid metabolism disorder are treated with statin + fibrate or statin + omega-3 fatty acids. In the absence of comparative studies, neither of these two treatments can be preferred to the other. It may be best to test both combinations and then continue with the one that is tolerated better and achieves a superior response (Parhofer, 2016).

When LDL cholesterol is low and triglyceride concentration is high, this is known as isolated hypertriglyceridemia. There could be an increase in total cholesterol. Isolated hypertriglyceridemia typically responds well to lifestyle changes, just like mixed hyperlipoproteinemia does. Still, it is impossible to know in advance how a particular patient would respond. There is no agreement on when to start a medication regimen because no compelling research has been released. When hypertriglyceridemia is unintentionally found in a person who is otherwise healthy, the threshold is higher; however, it is lower in patients who are at high risk of atherosclerosis. It is generally accepted that a fibrate can be administered if lifestyle modification strategies are implemented but the triglyceride level remains over 400 mg/dL (4.6 mmol/L). Fenofibrate (excellent tolerability) and gemfibrozil (positive endpoint trials; should not be taken with statins) appear to be the best options. If necessary, omega-3 fatty acids can also

be administered in combination. Since LDL cholesterol is frequently already quite low at the onset of isolated hypertriglyceridemia, statins are usually not particularly helpful. Patients with established atherosclerosis should take a low dose of statins (e.g., 10 mg atorvastatin or 20 mg simvastatin daily), regardless of their LDL cholesterol level (Parhofer, 2016).

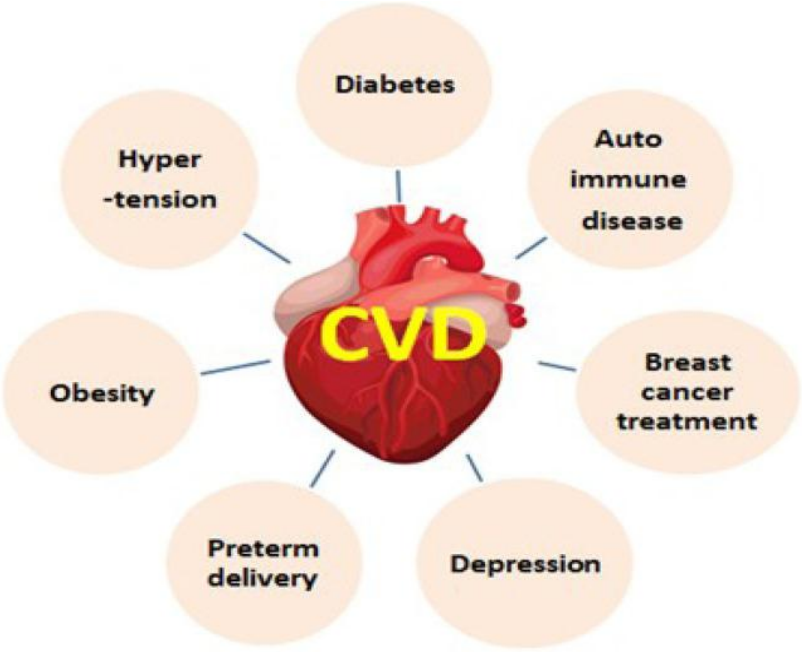

Fig. 2.2: showing various reasons for cardiovascular disorders by lipids storage related metabolic actions (Natesan and Kim, 2021).

Accurately categorizing lipid metabolism disorders is crucial. LDL hypercholesterolemia, hypertriglyceridemia, mixed hyperlipoproteinemia, and isolated reduction of HDL cholesterol are the different subtypes of them. It is possible for lipoprotein (a) increase to happen alone or in conjunction with other diseases of lipid metabolism.

- The degree of the lipid metabolism issue and the overall cardiovascular risk determine the need for and effectiveness of treatment.

- The LDL cholesterol level is the main outcome measure. According to the European guidelines, patients at high risk should have their LDL hypercholesterol levels lowered to a particularly high degree. Patients with mixed hyperlipoproteinemia and hypertriglyceridemia should prioritize changing their lifestyle..

- Because endpoint trials have shown beneficial effects, statins are widely used in medicine. Statin with ezetimibe is the only combination therapy that has demonstrated success in these studies; combinations containing fibrate and/or omega-3 fatty acids have not demonstrated any benefit as of yet. A limited number of patients may get PCSK9 antibodies. When atherosclerosis progresses, lipid apheresis may be necessary (Parhofer, 2016).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Centre

The study centres for this project were located at the Department of Pharmacology and Toxicology in the University of Benin (UNIBEN) and the University of Benin Teaching Hospital (UBTH), Benin City, Edo State, Nigeria.

3.2 Laboratory Animals Used for the Study

In the study, 60 albino wistar rats were used at the Department of Pharmacology and Toxicology in the University of Benin (UNIBEN). From the 60 albino wistrar rats, 54 of them were used for the positive controls that were administered with the drugs, while 6 were used for the negative controls that administered with water and feed only. On the 29th day, blood samples were collected from 47 albino wistar rats that survived after the daily drug administration of 28 days.

The blood samples were collected into plain containers and the serum samples were obtained for laboratory analysis in evaluating the changes in the lipid profile indices (Triglycerides, High density lipoprotein cholesterol, and Low density lipoprotein cholesterol) at the University of Benin Teaching Hospital (UBTH).

3.3 Ethical Approval Obtained from the Ethics Committee

Prior to the study, an ethical approval was requested from the Ethics Committee at the Faculty of Pharmacy under the supervision of Dr. Aghahowa Sylvester in the Department of Pharmacology and Toxicology, University of Benin, Benin City, Edo State, Nigeria.

A copy of the ethical approval has been attached to this project at the last page after the appendix section.

3.4 Materials, Apparatus, Reagents Used

The materials and apparatus used for the study include: Oral gastric tube, Weighing Balance, Spectrophotometer, Bucket Centrifuge, Glass pipettes, Syringes, Red cap plain containers, Micropipettes, Test tubes, Pasteur pipettes, automated pipette, Test tube rack, Refrigerator, Cotton wool, Water bath, Hand gloves, Permanent marker, Permanent marker, Methylated spirit, Chloroform, Albino rats, Albino rats feed and water.

The reagents that were utilized for this project were comprised of high analytical grade. They include Lipid profile estimation kit (Randox Laboratories LTD, UK).

3.5 Standard Dose of Recommended Anti-COVID-19 Drugs for Administration

Normally, research shows that the ideal weight range for albino rats is 150-250 grams. Values of 300 grams and above signals overweight rats and clinical implications. However, age is a physiological factor that can also lead to overweight.

Therefore, if 200 grams of albino rat is to receive a standard dose of 0.5ml drug administration,

1 gram of albino rat will receive;

(0.5 x 1) / 200 = 0.0025 ml

Hence, 0.0025 ml is the standard dose of drug to be administered per 1gram of albino rat weighed.

Cage 1: Chloroquine (CQ)

1 tail mark: 166 g weight x 0.0025 = 0.42 ml

2 tail mark: 173 g weight x 0.0025 = 0.43 ml

3 tail mark: 196 g weight x 0.0025 = 0.49 ml

4 tail mark: 136 g weight x 0.0025 = 0.34 ml

5 tail mark: 186 g weight x 0.0025 = 0.47 ml

6 tail mark: 148 g weight x 0.0025 = 0.37 ml

Cage 2: Hydroxylchloroquine (HCQ)

1 tail mark: 192 g weight x 0.0025 = 0.48 ml

2 tail mark: 161 g weight x 0.0025 = 0.40 ml

3 tail mark: 165 g weight x 0.0025 = 0.41 ml

4 tail mark: 140 g weight x 0.0025 = 0.35 ml

5 tail mark: 128 g weight x 0.0025 = 0.32 ml

6 tail mark: 160 g weight x 0.0025 = 0.40 ml

Cage 3: Ivermectin (IV)

1 tail mark: 171 g weight x 0.0025 = 0.43 ml

2 tail mark: 192 g weight x 0.0025 = 0.48 ml

3 tail mark: 164 g weight x 0.0025 = 0.39 ml

4 tail mark: 155 g weight x 0.0025 = 0.39 ml

5 tail mark: 164 g weight x 0.0025 = 0.41 ml

6 tail mark: 132 g weight x 0.0025 = 0.33 ml

Cage 4: Lopinavir/Retinavir (L.R)

1 tail mark: 122 g weight x 0.0025 = 0.31 ml

2 tail mark: 110 g weight x 0.0025 = 0.27 ml

3 tail mark: 152 g weight x 0.0025 = 0.38 ml

4 tail mark: 163 g weight x 0.0025 = 0.41 ml

5 tail mark: 126 g weight x 0.0025 = 0.32 ml

6 tail mark: 133 g weight x 0.0025 = 0.33 ml

Cage 5: Azithromycin (AZI)

1 tail mark: 118 g weight x 0.0025 = 0.29 ml

2 tail mark: 153 g weight x 0.0025 = 0.38 ml

3 tail mark: 184 g weight x 0.0025 = 0.46 ml

4 tail mark: 125 g weight x 0.0025 = 0.31 ml

5 tail mark: 144 g weight x 0.0025 = 0.36 ml

6 tail mark: 127 g weight x 0.0025 = 0.32 ml

Cage 6: Zinc (Zn) + Selinum (Se)

1 tail mark: 170 g weight x 0.0025 = 0.43 ml

2 tail mark: 143 g weight x 0.0025 = 0.36 ml

3 tail mark: 144 g weight x 0.0025 = 0.36 ml

4 tail mark: 134 g weight x 0.0025 = 0.34 ml

5 tail mark: 145 g weight x 0.0025 = 0.36 ml

6 tail mark: 125 g weight x 0.0025 = 0.31 ml

Cage 7: CQ + IV + L.R + AZI + Zn + Se

1 tail mark: 108 g weight x 0.0025 = 0.27 ml

2 tail mark: 131 g weight x 0.0025 = 0.33 ml

3 tail mark: 117 g weight x 0.0025 = 0.29 ml

4 tail mark: 149 g weight x 0.0025 = 0.37 ml

5 tail mark: 170 g weight x 0.0025 = 0.43 ml

6 tail mark: 130 g weight x 0.0025 = 0.33 ml

Cage 8: HCQ + AZI + L.R + IV + Zn + Se

1 tail mark: 153 g weight x 0.0025 = 0.38 ml

2 tail mark: 164 g weight x 0.0025 = 0.41 ml

3 tail mark: 178 g weight x 0.0025 = 0.45 ml

4 tail mark: 191 g weight x 0.0025 = 0.48 ml

5 tail mark: 159 g weight x 0.0025 = 0.39 ml

6 tail mark: 166 g weight x 0.0025 = 0.42 ml

Cage 9: IV + L.R + AZI + Zn + Sn

1 tail mark: 137 g weight x 0.0025 = 0.34 ml

2 tail mark: 146 g weight x 0.0025 = 0.37 ml

3 tail mark: 138 g weight x 0.0025 = 0.35 ml

4 tail mark: 160 g weight x 0.0025 = 0.40 ml

5 tail mark: 203 g weight x 0.0025 = 0.50 ml

6 tail mark: 138 g weight x 0.0025 = 0.35 ml

Cage 10: Water + Feed

1 tail mark: 144 g weight x 0.0025 = 0.36 ml

2 tail mark: 186 g weight x 0.0025 = 0.47 ml

3 tail mark: 178 g weight x 0.0025 = 0.45 ml

4 tail mark: 154 g weight x 0.0025 = 0.39 ml

5 tail mark: 161 g weight x 0.0025 = 0.40 ml

6 tail mark: 160 g weight x 0.0025 = 0.40 ml

3.6 Drugs Administration to the Albino Rats

Drug administration began on the 2nd of November, 2023 and was successfully completed on the 29th of November, 2023. For 28 days, daily administration of specific drugs were administered using the oral gastric tube. There were 10 cages. 9 of the cages were utilized for the positive control while 1 cage was utilized for the negative control (water and feed only, no drug administered). Before the drugs were administered, the weights of each of the albino rats were measured. On the day of the sample collection, the weights of the rats were also weighed.

3.7 Table Charts for Drug Administration and Daily Report

The drugs administration started 2nd November, 2023 and ended 29th November, 2023.

The table consists of the weight of the albino rats before the drug dosage was administered including the volume of the standard drugs given daily.

Where (m): male, (f): female

Weight of the albino rats before administration was measured in grams.

Volume of drug administered was given in millilitre volumes.

The table charts are in the appendix section.

3.8 Sample Collection

On the 29th day (30th of November, 2023), the albino rats were weighed and their weight were recorded. Thereafter, they were placed inside a chloroform covered container. Then, blood samples were taken from the lower abdominal region using a sterilized 23 gauge syringe 5ml needle. The blood samples were put inside well-labelled red-cap plain containers (without anticaogulant) to be used for laboratory analysis.

Fig. 3.1: showing the blood collection process of the albino rat.

Fig. 3.2: showing the blood collection process of the albino rat after utilizing chloroform.

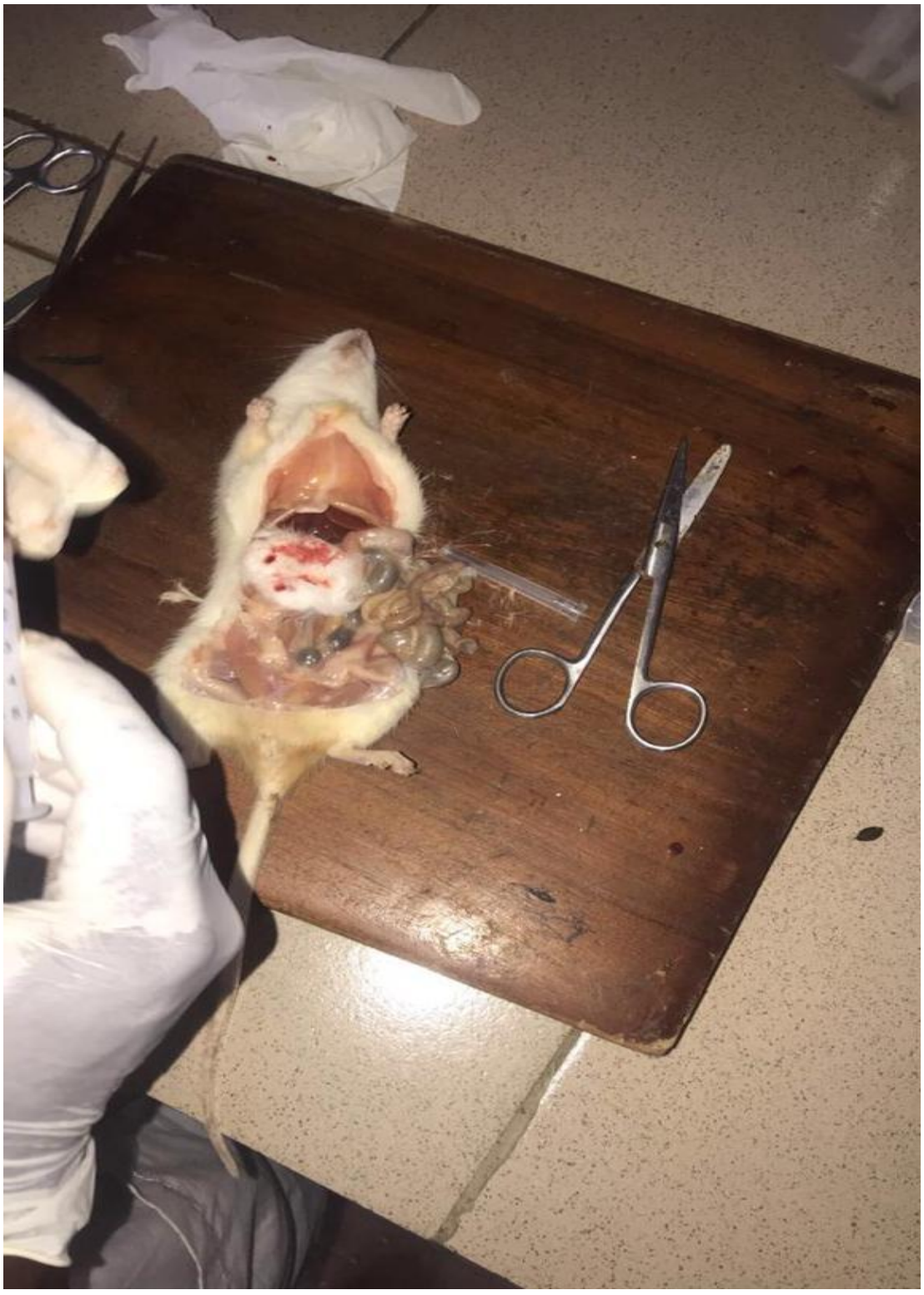

Fig. 3.3: showing the blood collection process of the albino rat using 5ml sterile syringe needle.

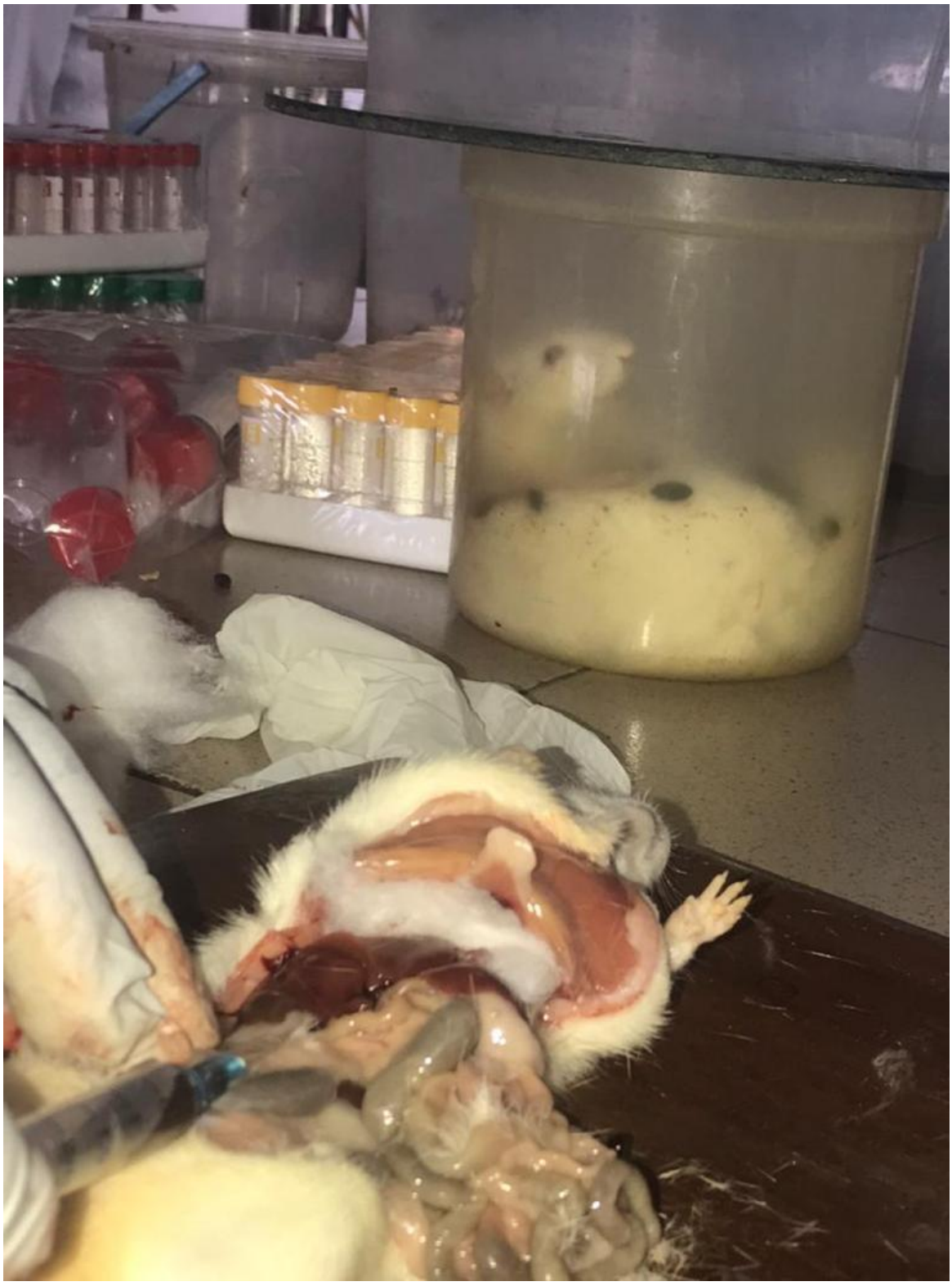

Fig. 3.4: showing the blood collection process, after which the sample collected into a sterile plain container for laboratory analysis.

3.9 Sample Extraction

The blood samples were spun using a bucket centrifuge for 4000 rpm for 5 minutes. The serum was extracted using an automated pipette. The serum was then transferred to the clean plain containers and further stored in a refrigerator.

3.10 Estimation of Serum Total Cholesterol Levels

Method: Enzymatic end point method (Tietz, 1990)

Principle: Total serum cholesterol is measured enzymatically in a series of coupled reactions that hydrolyse cholesteryl esters and oxidise 3-OH group of cholesterol. One of the reaction by-products, hydrogen peroxide (H_2O_2) is measured quantitatively in a peroxidase catalysed reaction that produces a colour (pink). Absorbance is read at 500nm. The intensity of the colour is proportional to the cholesterol concentration.

The reaction follows this pattern:

Cholesterol ester + H_20 $\xrightarrow{\text{cholesterol ester hydrolase}}$ Cholesterol + fatty acids

Cholesterol + O_2 $\xrightarrow{\text{cholesterol oxidase}}$ Cholest-4-ene-3one + H_2O_2

$2H_2O_2$ $\xrightarrow{\text{peroxidase}}$ 4-aminophenazone + $4H_2O$ + 4-(p-benzoquinone-monoimino)-phenazone

Procedure:

Frozen plasma was thrawed and kept at 37 degree celsius prior to analysis.

Below is the protocol table:

Blank Tubes	Blank (µl)	Standard (µl)	Test (µl)
Reagent	1000	1000	1000
Sample	-	-	10
Standard	-	10	-
Distilled water	10	-	-
Total mixture	1010	1010	1010

The total mixture for each tube (for blank, standard, and test) was incubated at a room temperature for 10 minutes. The absorbance was read at 500nm against reagent blank and was further used to obtain the concentration of the total cholesterol using the calculation below.

Calculation:

To obtain the total cholesterol concentration (mg/dl) of the serum samples using a manual method, the formula below is used:

Total Cholesterol Concentration= (Absorbance of test / Absorbance of Standard) x Concentration of Standard.

Reference Range:

150 – 200mg/dL

3.11 Estimation of Serum Triglycerides

Method: GPO-PAP (Tietz, 1990)

Principle: Triglycerides are measured enzymatically in plasma using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol. Glycerol is then oxidized using glycerol oxidase and hydrogen peroxide (H_2O_2), one of the products of the reaction is measured as described for cholesterol. Absorbance is measured at 500nm.

The reaction follows this pattern:

Triglycerides + $3H_20$ $\xrightarrow{\text{lipase}}$ glycerol + fatty acids

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

Glycerol-3-phosphate + glycerolphosphate-oxidase $\longrightarrow$ dihydroacerone phosphate (DHAP) + H_2O_2

Peroxidase + H_2O_2 + 4-aminophenazone + 4-chlorophenol $\longrightarrow$ 4-(p-bezoquinone-monoimino)-phenazone + $2H_2O$

Procedure:

Frozen plasma was thrawed and kept at room temperature at 37 degree celsus prior to analysis.

Below is the protocol table:

Blank Tubes	Blank (µl)	Standard (µl)	Test (µl)
Reagent (R1)	1000	1000	1000
Sample	-	-	10
Standard	-	10	-
Distilled water	10	-	-
Total mixture	1010	1010	1010

The total mixture for each tube (for blank, standard, and test) was incubated at a room temperature for 10 minutes. The absorbance was read at 500nm against reagent blank and was further used to obtain the concentration of the triglyceride using the calculation below.

Calculation:

To obtain the triglyceride concentration (mg/dl) of the serum samples using a manual method, the formula below is used:

Triglyceride Concentration= (Absorbance of test / Absorbance of Standard) x Concentration of Standard

Reference Range:

≤150mg/dL

3.12 Estimation of Serum High Density Lipoprotein Cholesterol (HDLc)

Method: Enzymatic End Point Method (Teitz, 1990).

Principle

Lipoproteins present in the specimen (LDL, VLDL and Chylomicrons) are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. This renders them non-reactive and effectively excluded from the assay. Thus, after centrifugation, only HDL-cholesterol is detected.

Procedure:

1. Precipitation:

Precipitate into centrifuge tubes

	Micro (µl)	**Semi micro(µl)**
Sample/Standard	500	200
Precipitant (R1)	1000	-
Diluted precipitant (R1)	-	500
Total mixture	1500	700

The total mixture for each tube (for blank, standard, and test) was incubated at a room temperature and allowed to stand at room temperature after which it was centrifuged at a time duration of 10 minutes at 4000rpm.

Within two hours, the clear supernatant was collected, and the CHOD-PAP technique was used to calculate the cholesterol content.

2. Cholesterol (Chod-Pap) Assay

Blank Tubes	Blank (µl)	Standard (µl)	Test (µl)
Reagent	1000	1000	1000
Sample	-	-	100
Standard	-	100	-
Distilled water	100	-	-
Total mixture	1100	1100	1100

The total mixture for each tube (for blank, standard, and test) was incubated for 10 minutes at room temperature. The absorbance was read at 500nm against water blank.

Calculation:

To obtain the HDL-c concentration (mg/dl) of the serum samples using a manual method, the formula below is used:

HDL-c Concentration= (Absorbance of test / Absorbance of Standard) x Concentration of Standard

Reference Range:

35 – 65mg/dL

3.13 Estimation of Low Density Lipoprotein Cholesterol (LDL-c)

LDL-c is calculated from the measured values of total cholesterol, triglycerides, and HDL-cholesterol:

LDL-c = TC – (HDL + TG/5)

This is also known as the Freidwald equation (Freidwald *et al.,* 1972).

Reference Range:

≤150mg/dL

3.14 Atherogenic Indices

Cardiac Risk Ratio (CRR) = TC/ HDL-c

Atherogenic Coefficient (AC) = (TC – HDL-c)/HDL-c

Atherogenic Index of Plasma (AIP) = log (TG/HDL-c)

Examination of the data from cohorts with various atherogenic risk has revealed highly significant correlation between FERHDL and logarithmically transformed ratio TG/HDL-C as well as the LDL particle size. Based on these findings, we suggest that Log(TG/HDL-C) may be used as a simple, readily calculated parameter about atherogenicity of plasma lipoproteins (Dobiášová and Frohlich, 2001).

3.15 Statistical Analysis

The data obtained from the study were analyzed using SPSS (Statistical Package for Social Sciences) version 27. The Standard Error of Mean were represented as mean ± SEM for tests and controls. ANOVA (Analysis of Variance) was also utilized in the comparison of the data at a confidence interval of 95% ($P<0.05$).

CHAPTER FOUR

RESULTS

Table 4.1 presents the mean values (± standard error) of Total Cholesterol (TC), Triglycerides (TG), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), and Weight of rats in the various treatment groups (A-J). The Control group (A) shows TC at 163.67 mg/dl, TG at 85.33 mg/dl, HDL at 47.00 mg/dl, LDL at 94.33 mg/dl, and Weight at 163.83 g. The statistical analysis indicates significant differences ($p \leq 0.05$) in HDL levels between the control group and the treatment group H. Additionally, significant differences were found in Weight between the control group and treatment groups D, H, and F, suggesting potential effects of these treatments on lipid profiles and weight. However, other comparisons did not show significant differences. The table also provides post hoc analysis results, which further detail the specific comparisons and significance levels between groups.

Keys:

*= Significant at $p \leq 0.05$

SE= Standard Error

HDL= High density lipo-protein

TG= Triglycerides

LDL= Low density lipo-protein

TC= Total Cholesterol

Table 4.1: showing the High Density Lipo-protein, Triglycerides, Low Density Lipo-protein, Total Cholesterol and Weight before treatment of the Treatment groups

Treatment Groups	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Weight (g)
	Mean±SEM				
Control (**A**)	163.67±15.218	85.33±7.817	47.00±2.422	94.33±15.682	163.83 ±6.337
Azithromycin (**B**)	143.17±12.411	81.00±6.836	40.33±3.801	86.67±12.002	141.83 ±9.965
Choloroquine (**C**)	144.83±20.542	72.83±11.887	50.67±3.323	82.83±16.316	167.50 ±9.237
Hydroxylcholoroquine (**D**)	123.00±15.593	76.17±9.995	49.33±3.313	58.50±12.019	157.67 ±9.021
Ivermectin (**E**)	138.20±21.627	69.40±12.420	48.60±5.134	75.80±19.088	155.20 ±6.583
Lopinavir/Retinavir (**F**)	151.83±8.183	60.33±10.343	40.67±3.938	99.00±8.189	134.33 ±8.061
Zinc/selenium (**G**)	136.33±6.776	50.50±4.372	37.83±1.447	95.17±10.731	143.50 ±6.158
CQ + IV + L.R + AZI + Zn + Se (**H**)	151.00±7.000	65.00±17.521	30.67±5.608	107.33±3.528	143.25 ±5.588
HCQ + AZI + L.R + IV + Zn + Se (**I**)	153.20±7.446	66.40±12.640	38.20±4.521	101.80±7.165	134.75 ±12.89 3
IV + L.R + AZI + Zn + Sn (**J**)	148.25±5.677	10.392±5.196	41.50±4.113	95.50±6.538	170.40 ±6.501
F	0.671	1.268	2.539	1.207	2.582
P	0.731	0.282	0.020*	0.316	0.018*
Post Hoc					
A vsB	.284	.746	.183	.659	.054*
A vsC	.324	.351	.460	.509	.743
A vsH	.587	.218	.010*	.542	.024*
A vs I	.600	.181	.096	.682	.577
A vs D	.037*	.493	.638	.044*	.582
A vs J	.469	.063	.323	.952	.105
A vs E	.206	.258	.758	.311	.463
A vs F	.534	.066	.205	.788	.011*
A vs G	.155	.012*	.069	.962	.075

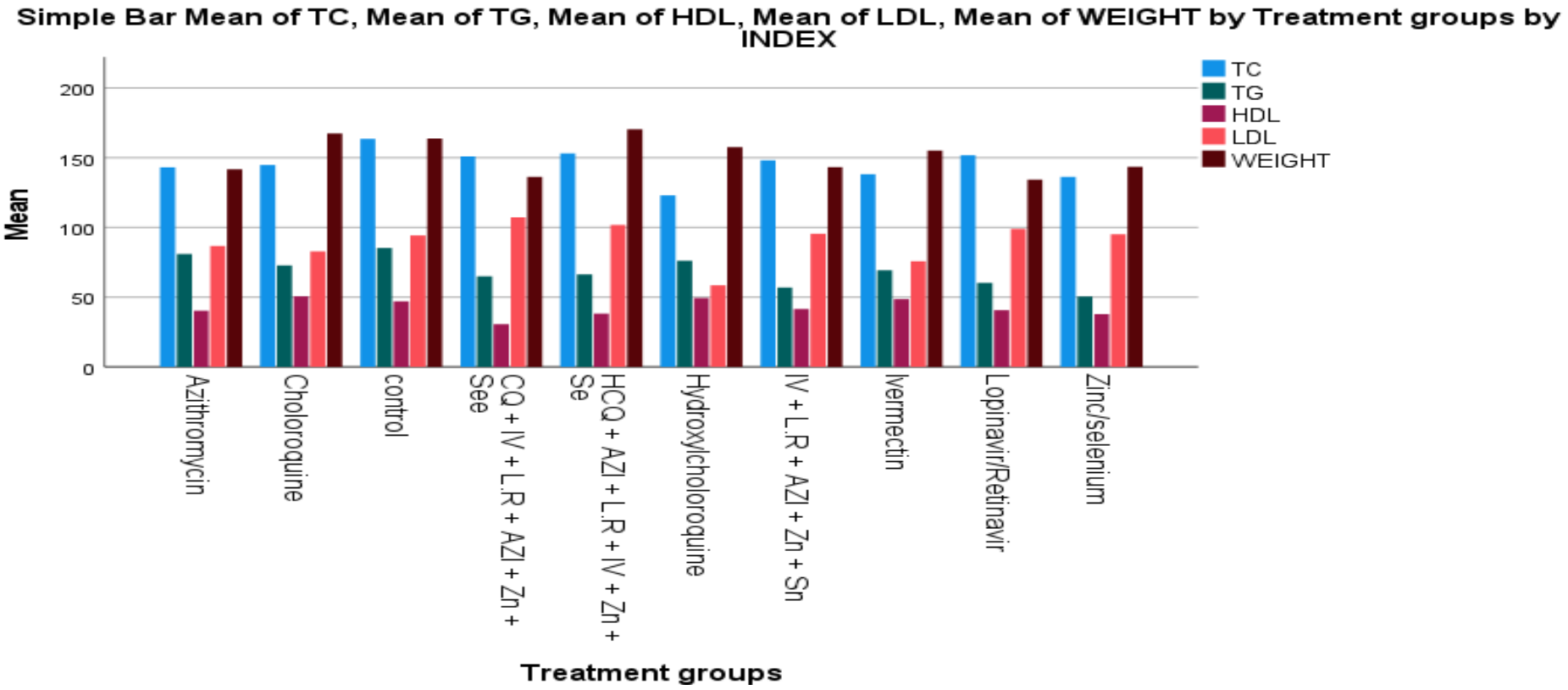

Fig. 4.1: showing the simple bar chart mean of TC, mean of HDL, mean of LDL and mean of Weight of treatment by INDEX.

Table 4.2 presents a comprehensive overview of the Atherogenic Index, Cardiac Risk Ratios, and Atherogenic Coefficient across various treatment groups. Each treatment group is denoted by a letter (A-J), with corresponding values indicating the Mean±SE for each parameter. Notably, the Atherogenic Index ranges from 0.1191±0.038 to 0.3141±0.085, with treatment group J demonstrating the lowest value and group D exhibiting the highest. Similarly, Cardiac Risk Ratios vary from 2.4818±0.21158 to 5.2648±0.96836, with group D again displaying the highest ratio and group F the lowest. Additionally, the Atherogenic Coefficient ranges from 1.4818±0.21158 to 4.2648±0.96836, further illustrating the variability in cardiovascular risk across treatment modalities. The significance of these findings is underscored by the Post hoc analysis, which highlights significant differences between certain treatment pairs, as denoted by asterisks (*). Specifically, comparisons between treatment group C and groups A, D, and J reveal statistically significant differences in Cardiac Risk Ratios and Atherogenic Coefficients.

Table 4.2: showing the Atherogenic Index, Cardiac Risk Ratios and Atherogenic Coeficient of the various treatment groups.

Treatment Groups	Atherogenic Index	Cardiac Risk Ratios	Atherogenic Coeficient
		Mean±SEM	
Azithromycin (A)	.3044±.025	3.6770±.45926	2.6770±.45926
Choloroquine (B)	.1368±.049	2.8669±.35983	1.8669±.35983
Control (C)	.2523±.036	3.5639±.42575	2.5639±.42575
CQ + IV + L.R + AZI + Zn + Se (D)	.3141±.085	5.2648±.96836	4.2648±.96836
HCQ + AZI + L.R + IV + Zn + Se (E)	.2251±.074	4.2444±.54830	3.2444±.54830
Hydroxylcholoroquine (F)	.1679±.074	2.4818±.21158	1.4818±.21158
IV + L.R + AZI + Zn + Sn (G)	.1388±.075	3.6815±.41815	2.6815±.41815
Ivermectin (H)	.1347±.089	2.8991±.38206	1.8991±.38206
Lopinavir/Retinavir (I)	.1483±102	3.9049±.41515	2.9049±.41515
Zinc/selenium (J)	.1191±.038	3.6284±.22428	2.6284±.22428
F	1.100	2.835	2.835
P	0.383	0.010*	0.010*
Post hoc			
C vs A	.565	.841	.841
C vs B	.206	.219	.219
C vs D	.578	.017*	.017*
C vs E	.775	.252	.252
C vs F	.354	.060	.060
C vs G	.266	.852	.852
C vs H	.220	.263	.263
C vs I	.254	.545	.545
C vs j	.146	.909	.909

*=p value significant at ≤0.05; SE= Standard Error

Table 4.3 presents the pre- and post-treatment weights across various treatment groups. Each group received a different therapeutic regimen for a particular condition. The table indicates the mean weight in grams along with the standard error (SE) before and after treatment, as well as the corresponding p-values. Before treatment, the weight ranged from 134.33g to 170.40g across different groups. After treatment, the weights varied from 181.67g to 207.20g. Notably, Chloroquine (CQ), Hydroxychloroquine (HQ), Ivermectin (IV), Lopinavir/Retinavir (LR), Azithromycin (AZI), and Zinc (ZN) treatments showed statistically significant changes in weight, as indicated by p-values of .032*, .001*, .001*, .000*, .046*, and .000* respectively, where * denotes significance at $p \leq 0.05$. The combined treatment of CQ, IV, LR, AZI, and ZN also demonstrated a significant impact on weight with a p-value of .014*. However, the combination of HCQ, AZI, LR, IV, and ZN did not yield a statistically significant change in weight ($p = .240$).

Table 4.3: showing weight before and after treatment

Treatment groups	Weight (g) Before Treatment	Weight (g) After Treatment	P value
	Mean ± SEM		
Chloroquine (CQ)	167.50±9.237	204.83±10.521	.032*
Hydroxylcholoroquine (HQ)	157.67±9.021	188.00±7.958	.001*
Ivermectin (IV)	155.20±6.583	207.20±11.993	.001*
Lopinavir/Retinavir (LR)	134.33±8.061	181.67±7.982	.000*
Azithromycin (AZI)	153.50±11.144	198.17±16.040	.046*
Zinc (ZN)	143.50±6.158	194.17±8.252	.000*
CQ+IV+LR+AZI+ZN+SE	136.33±18.095	192.33±23.905	.014*
HCQ+AZI+LR+IV+ZN+SE	170.40±6.501	190.60±14.521	.240
IV+LR+AZI+ZN+SN	143.25±5.588	193.75±6.169	.010*

*=p value significant at ≤0.05; SE= Standard Error

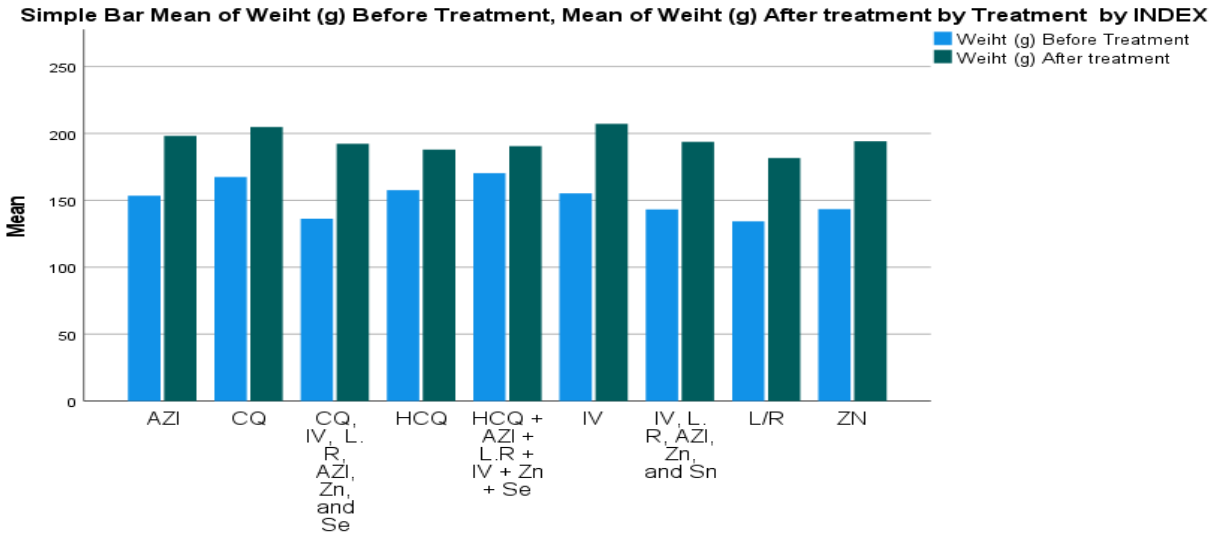

Fig. 4.2: Showing the simple bar chart of weight (g) before treatment and mean of weight (g) after treatment by INDEX.

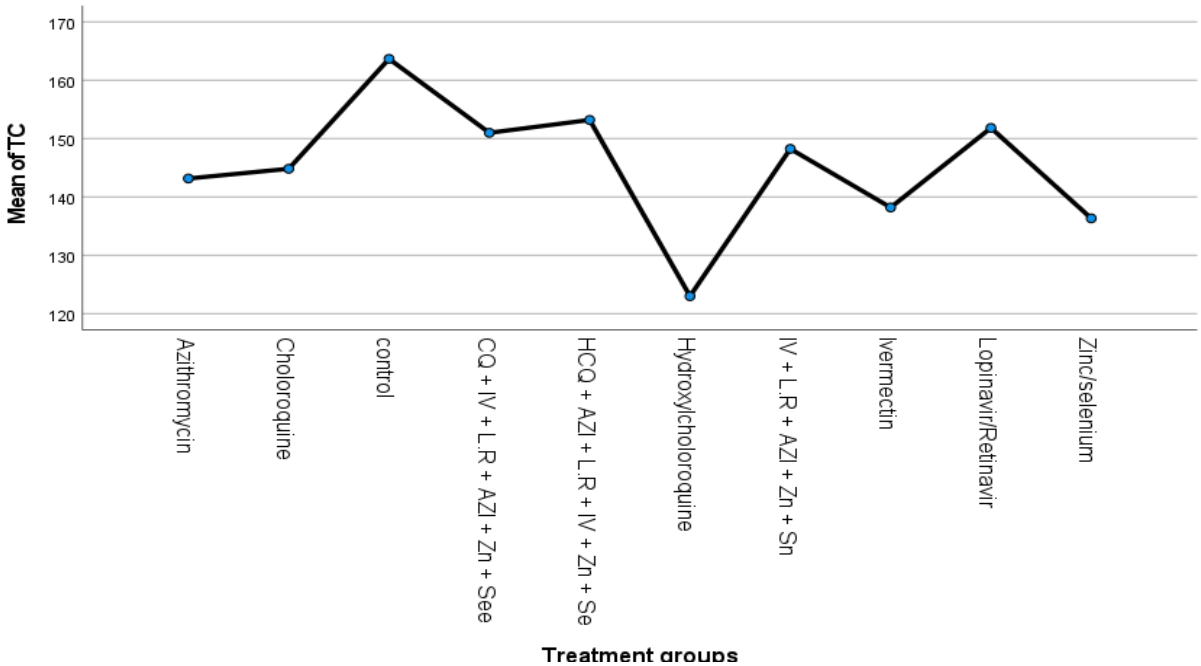

Fig. 4.3: Showing the mean of TC of the treatment groups.

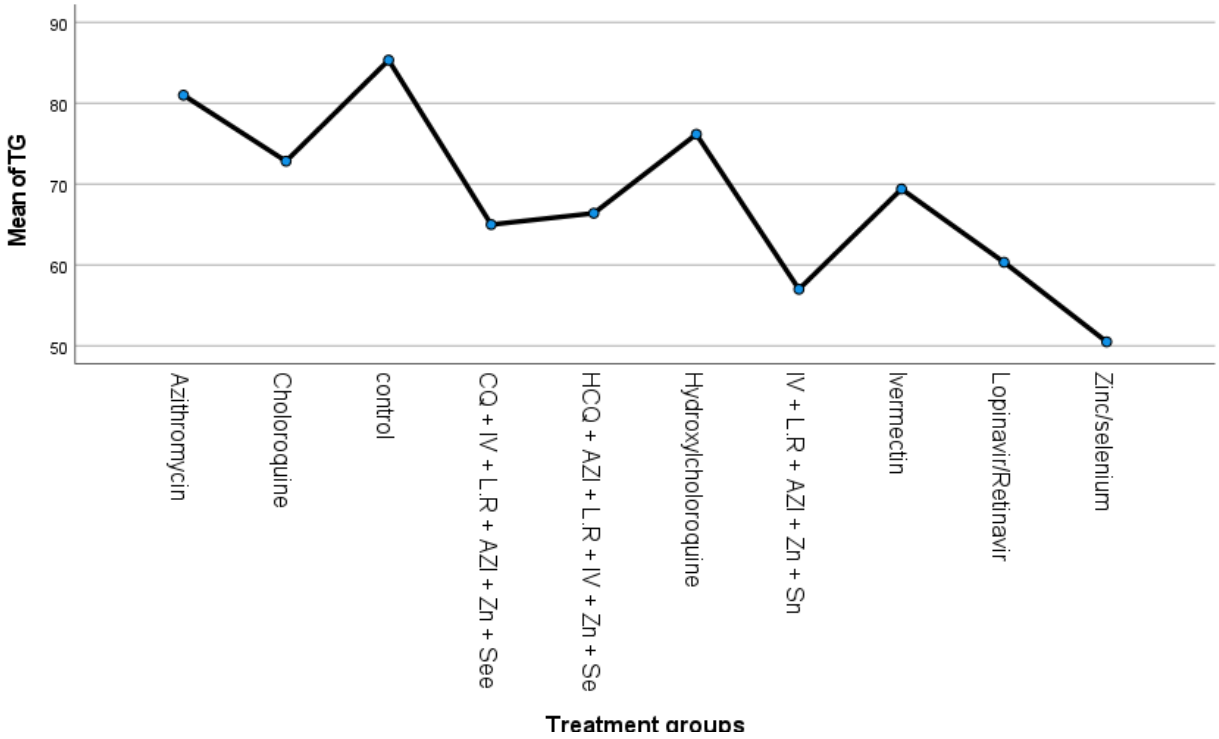

Fig. 4.4: Showing the mean of TG of the treatment groups.

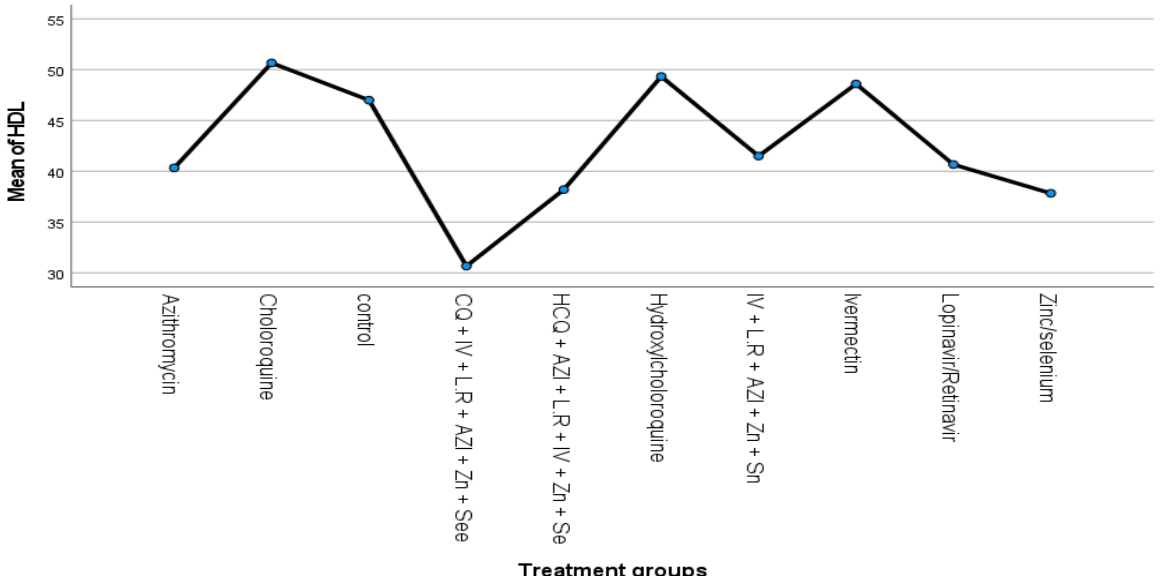

Fig. 4.5: Showing the mean of HDL of the treatment groups.

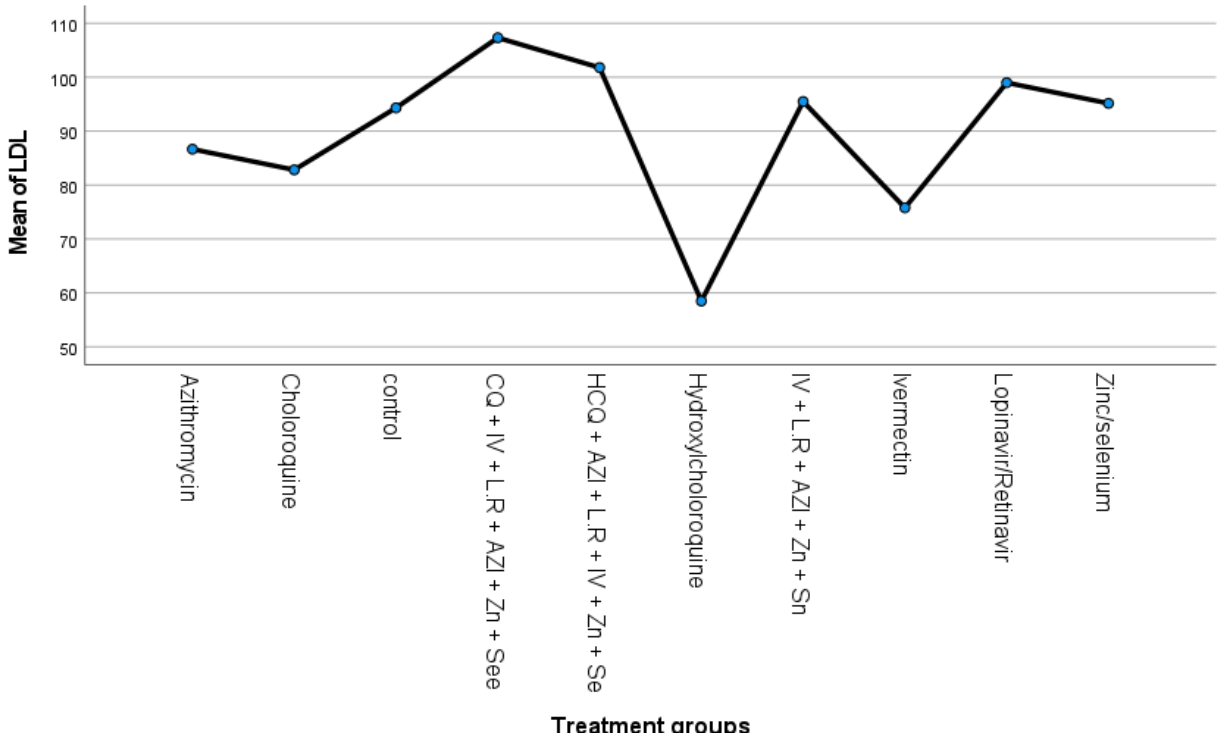

Fig. 4.6: Showing the mean of LDL of the treatment groups.

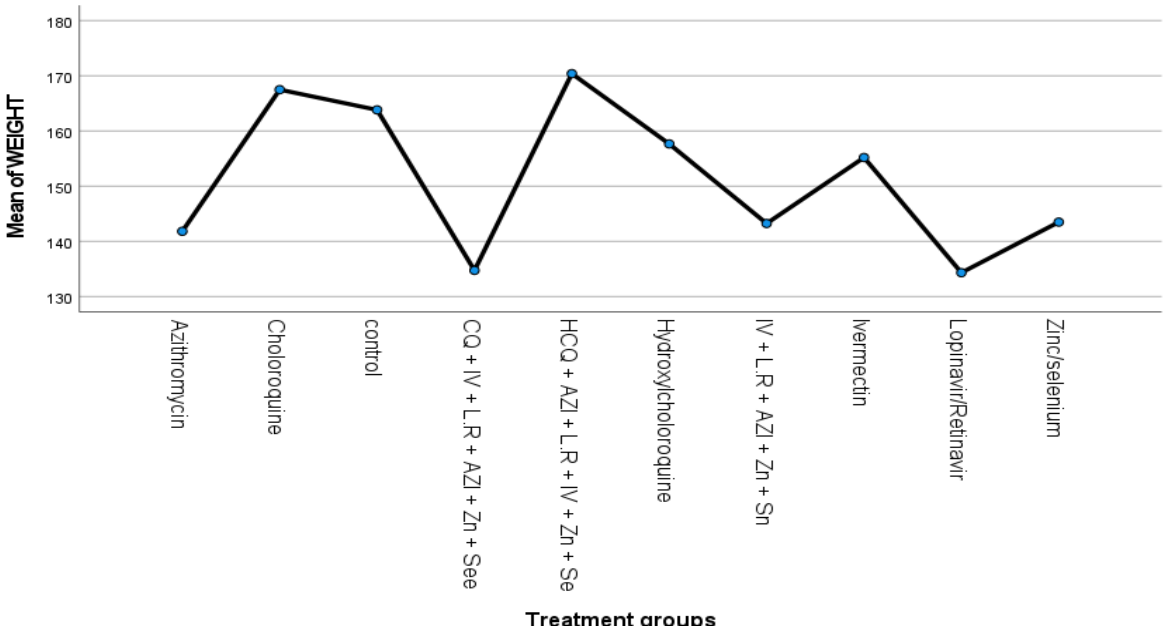

Fig. 4.7: Showing the mean of weight of the treatment groups.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

The impact of various medications, including Hydroxychloroquine (HCQ), Chloroquine (CQ), Ivermectin (IV), Lopinavir/ritonavir (L.R), Azithromycin (AZI), Zinc (Zn), and Selenium (Se), on lipid profile and weight is a subject of increasing interest in medical research. Hydroxychloroquine and chloroquine, traditionally used in treating malaria and autoimmune diseases, have been implicated in modulating lipid metabolism, potentially affecting cholesterol levels (Sager and Koenig, 2017). Similarly, Ivermectin, primarily known for its antiparasitic properties, and Lopinavir/ritonavir, used in HIV treatment, may have implications for lipid profiles. Azithromycin, an antibiotic, has been linked to weight changes and lipid alterations in some studies. Additionally, micronutrients like Zinc and Selenium are essential for metabolic processes and may influence weight and lipid profiles (Ashmawy *et al*., 2022).

The study examined the effects of various treatments on lipid profile and weight in a control group and multiple treatment groups. Notably, hydroxychloroquine (HCQ) demonstrated a significant reduction in total cholesterol (TC) levels compared to the control group, while the combination treatment including chloroquine (CQ), IV, lopinavir/ritonavir (L.R), AZI, zinc (Zn), and selenium (Se) resulted in a significant decrease in HDL levels and weight compared to the control.

Hydroxychloroquine (HCQ) has been shown to significantly reduce total cholesterol (TC) levels in patients with systemic lupus erythematosus (SLE) and lupus nephritis. In a study by Gheet *et al*., (2023) HCQ treatment resulted in a significant decrease in cholesterol levels after 12 months of treatment (Gheet *et al*., 2023). Another study by Ye *et al*. (2023) found that HCQ treatment in SLE patients led to a reduction in lipids, including cholesterol (Ye *et al*., 2023). Additionally, a meta-analysis by Wakiya *et al*. (2020) showed that the use of HCQ in pregnant women with SLE and/or antiphospholipid syndrome (APS) resulted in a decreased risk of high lupus activity, and preeclampsia, which is associated with dyslipidemia (Wakiya *et al*., 2020). These findings suggest that HCQ has a beneficial effect on cholesterol levels in patients with SLE and lupus-related pregnancy complication. Hydroxychloroquine (HCQ) has been shown to reduce total cholesterol (TC) levels through various molecular mechanisms. One possible mechanism is the drug's effect on autophagy-related proteins, such as ribosyldihydronicotinamide dehydrogenase (NQO2) and transport protein Sec23A (SEC23A), which are involved in cholesterol metabolism (Sarkar and Singh, 2023).

Additionally, HCQ may regulate the expression of galectin-8 (LGALS8) and mitogen-activated protein kinase 8 (MAPK8), which can impact cholesterol levels (Zhao *et al*., 2022). Another potential mechanism is HCQ's interaction with G-quadruplex (Gq) structures in the telomere region associated with cancer. HCQ preferentially binds to the hybrid form of Gq, stabilizing it and potentially affecting cholesterol metabolism (Costa *et al*., 2022).

Furthermore, HCQ's glucocorticoid-sparing effect and reduction in systemic inflammation may contribute to its ability to lower TC levels (Sarkar *et al*., 2022).

The study examined cardiac risk ratios and atherogenic coefficients across various treatment groups. Chloroquine (CQ) demonstrated significantly lower cardiac risk ratios ($p<0.05$) and atherogenic coefficients ($p<0.05$) compared to the control group, indicating a potential reduction in cardiovascular risk and atherogenic potential. Hydroxychloroquine (HCQ) also exhibited significantly lower cardiac risk ratios ($p<0.05$) and atherogenic coefficients ($p<0.05$) than the control group. However, the combination treatment including CQ, IV, lopinavir/ritonavir (L.R), azithromycin (AZI), zinc (Zn), and selenium (Se) displayed significantly higher cardiac risk ratios and atherogenic coefficients ($p<0.05$) compared to the control, indicating a potential increase in cardiovascular risk and atherogenic potential with this combination therapy. One possible mechanism is the increase in systemic inflammation caused by antimicrobial treatment (Schenkein and Loos, 2013). Periodontal diseases, which are associated with systemic inflammation, have been linked to higher levels of pro-inflammatory cytokines and high-sensitivity C-reactive protein. Another mechanism could be the promotion of dyslipidemia by antimicrobial treatment, leading to increases in pro-inflammatory lipid classes and subclasses (Sager and Koenig, 2017). Additionally, cross-reactive systemic antibodies stimulated by bacteria at sites distant from the oral cavity may interact with the atheroma and promote inflammation (Schenkein and Loos, 2013).

In this study, Chloroquine (CQ), hydroxychloroquine (HQ), ivermectin (IV), lopinavir/ritonavir (LR), azithromycin (AZI), and zinc (ZN) treatments all showed significant increases in weight after treatment compared to baseline ($p<0.05$). The combination treatment with CQ, IV, LR, AZI, ZN, and selenium (SE) also demonstrated a significant increase in weight post-treatment ($p<0.05$). Conversely, the combination treatment including hydroxychloroquine, AZI, LR, IV, ZN, and SE did not result in a significant change in weight ($p>0.05$).

A study found that CQ treatment resulted in weight gain in malnourished children, suggesting that it may have a role in promoting weight gain (Ashmawy *et al*., 2022a). Another study showed that CQ treatment in high-fat diet-induced obese rats reduced weight gain and improved lipid profile (Blignaut *et al*., 2019). However, a different study found that CQ treatment, both acutely and chronically, resulted in myocardial dysfunction and decreased heart function in rats (Angelakis *et al*., 2014). Additionally, a study examining the safety of CQ use in pregnant women found no clear increased risk of weight gain in infants exposed to CQ in utero (Bérard *et al*., 2021). CQ and HQ have been shown to inhibit lipogenesis-related enzymes, such as sterol regulatory element-binding protein (SREBP)-1c, fatty acid synthase (FAS), and acetyl-CoA carboxylase (ACC), in liver and adipose tissue, leading to reduced weight gain (Ashmawy *et al*., 2022b; Phan *et al*., 2023). However, this study reveals a significant weight gain after treatment with CQ and HQ respectively.

Additionally, CQ has been found to affect lysosomal function and protein clearance, resulting in the build-up of autophagic vacuoles and protein aggregates, which are associated with CQ-induced myopathy (Panizzutti *et al*., 2021).

5.2 CONCLUSION

The study's findings clarified how different medications affected atherogenic coefficients, weight, cardiac risk ratios, and lipid profiles. Hydroxychloroquine (HCQ) demonstrated a significant reduction in total cholesterol levels, aligning with previous findings indicating its potential to modulate lipid metabolism beneficially. However, the combination treatment involving chloroquine (CQ), ivermectin (IV), lopinavir/ritonavir (L.R), azithromycin (AZI), zinc (Zn), and selenium (Se) exhibited a concerning increase in cardiac risk ratios and atherogenic coefficients, suggesting a potential exacerbation of cardiovascular risk factors with this combination therapy. Mechanistically, antimicrobial treatment may induce systemic inflammation and dyslipidemia, contributing to the observed effects. Moreover, while both CQ and HCQ treatments led to significant weight gain, contrary to some previous findings, their mechanisms of action on weight regulation remain complex and warrant further investigation.

5.3 RECOMMENDATIONS

1) There is need for greater caution in the use of these drugs, and careful monitoring of patients taking them is recommended. Ultimately, alternative treatments should be considered whenever possible to minimize the risks of adverse effects.

2) The long-term impacts of prescribed medications on lipid profile should be investigated further.

3) Examination of the effects of these drugs on other organ systems should be done.

4) Research into alternative treatments for the conditions for which these drugs are recommended should be done.

5) Evaluation of the potential benefits and risks of recommended drugs, including the cost-benefit analysis.

6) Development of safer and more effective drugs to treat the conditions for which these drugs are currently recommended.

These suggestions may aid in enhancing patient outcomes and lowering the possibility of negative side effects from using the suggested medications.

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APPENDIX

Raw data of results from biochemical analysis of lipids of the albino rats

LIPIDS										
TC	CON	CQ	HQ	IV	L/R	AZ	ZN/SE	7	8	9
1	181	131	193	151	127	112	130	165	134	159
2	226	233	138	118	137	110	108	144	160	157
3	121	100	86	217	166	140	146	144	178	140
4	153	171	111	106	139	168	140		148	137
5	166	102	103	99	177	142	137		146	
6	135	132	107		165	187	157			
TG	CON	CQ	HQ	IV	L/R	AZ	ZN/SE	7	8	9
1	61	63	105	46	39	69	49	100	40	46
2	108	127	77	65	35	75	46	46	62	60
3	97	63	33	101	64	61	37	49	114	70
4	64	68	77	95	44	106	57		52	52
5	85	40	93	40	84	95	68		64	
6	97	76	72		96	80	46			
HDL	CON	CQ	HQ	IV	L/R	AZ	ZN/SE	7	8	9
1	47	52	63	40	35	32	40	39	31	52
2	44	53	47	38	59	37	37	33	26	32
3	54	50	51	62	39	37	36	20	42	40
4	37	52	52	43	40	52	32		40	42
5	50	36	43	60	40	52	41		52	
6	50	61	40		31	32	41			
LDL	CON	CQ	HQ	IV	L/R	AZ	ZN/SE	7	8	9
1	90	66	109	102	84	66	80	106	95	98
2	160	155	76	67	71	58	62	102	122	113
3	48	57	28	135	114	91	103	114	113	86
4	103	105	44	44	90	95	137		98	85
5	99	58	41	31	120	71	82		81	
6	66	56	53		115	139	107			

The daily drug administration (28 Days) of Chloroquine (CQ) to the Albino Rats

Cage 1: Chloroquine (CQ)

Albino rats	Weight	Volume	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1 tail mark	166g(m)	0.42ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
2 tail mark	173g(f)	0.43ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
3 tail mark	196g(m)	0.49ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
4 tail mark	136g(m)	0.34ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
5 tail mark	186g(f)	0.47ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
6 tail mark	148g(m)	0.37ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

*6 tail mark albino rat died on the 18th of November, 2023 and was replaced immediately.

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The daily drug administration (28 Days) of Hydroxylchloroquine (HCQ) to the Albino Rats

Cage 2: Hydroxylchloroquine (HCQ)

Albino rats	Weight	Volume	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1 tail mark	192g(f)	0.48ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
2 tail mark	161g(m)	0.40ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
3 tail mark	165g(f)	0.41ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
4 tail mark	140g(m)	0.35ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
5 tail mark	128g(m)	0.32ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
6 tail mark	160g(m)	0.40ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

The daily drug administration (28 Days) of Ivermectin (IV) to the Albino Rats

Cage 3: Ivermectin (IV)

Albino rats	Weight	Volume	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1 tail mark	171g(m)	0.43ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
2 tail mark	192g(m)	0.48ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√		
3 tail mark	154g(m)	0.39ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
4 tail mark	155g(m)	0.39ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
5 tail mark	164g(m)	0.41ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
6 tail mark	132g(m)	0.33ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

*2 tail marked albino rat died on the 27th of November, 2023.

The daily drug administration (28 Days) of Lopinavir/Retinavir to the Albino Rats

Cage 4: Lopinavir/Retinavir (L.R)

Albino rats	Weight	Volume	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1 tail mark	122g(m)	0.31ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
2 tail mark	110g(m)	0.28ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
3 tail mark	152g(m)	0.38ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
4 tail mark	163g(m)	0.41ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
5 tail mark	126g(m)	0.32ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
6 tail mark	133g(m)	0.33ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

The daily drug administration (28 Days) of Azithromycin (AZI) to the Albino Rats

Cage 5: Azithromycin (AZI)

Albino rats	Weight	Volume	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1 tail mark	188g(m)	0.30ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
2 tail mark	153g(m)	0.38ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
3 tail mark	184g(f)	0.46ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
4 tail mark	125g(m)	0.31ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
5 tail mark	144g(m)	0.36ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
6 tail mark	127g(m)	0.32ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

The daily drug administration (28 Days) of Zinc (Zn) and Selinum (Se) to the Albino Rats

Cage 6: Zinc (Zn) + Selinum (Se)

Albino rats	Weight	Volume	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1 tail mark	170g(f)	0.43ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
2 tail mark	143g(m)	0.36ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
3 tail mark	144g(m)	0.36ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
4 tail mark	134g(f)	0.34ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
5 tail mark	145g(m)	0.36ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
6 tail mark	125g(m)	0.31ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

The daily drug administration (28 Days) of CQ, IV, L.R, AZI, Zn, and Se to the Albino Rats

Cage 7: CQ + IV + L.R + AZI + Zn + Se

Albino rats	Weight	Volume	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1 tail mark	108g(m)	0.27ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
2 tail mark	131g(m)	0.33ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
3 tail mark	117g(m)	0.29ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√										
4 tail mark	149g(m)	0.37ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√			
5 tail mark	170g(m)	0.43ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
6 tail mark	130g(m)	0.33ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√											

*3 tail marked albino rat died on the 19th of November, 2023.

*4 tail marked albino rat died on the 26th of November, 2023.

*6 tail marked albino rat died on the 18th of November, 2023.

The daily drug administration (28 Days) of HCQ, AZI, L.R, IV, Zn, and Se to the Albino Rats

Cage 8: HCQ + AZI + L.R + IV + Zn + Se

Albino rats	Weight	Volume	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1 tail mark	153g(m)	0.38ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
2 tail mark	164g(f)	0.41ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
3 tail mark	178g(f)	0.45ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
4 tail mark	191g(f)	0.48ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
5 tail mark	159g(f)	0.40ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√				
6 tail mark	166g(f)	0.42ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

*2 tail marked albino rat died on the 15th of November, 2023 and was replaced.

*5 tail marked albino rat died on the 25th of November, 2023 and was not replaced.

*6 tail marked albino rat displayed paleness and neck bending.

The daily drug administration (28 Days) of IV, L.R, AZI, Zn, and Sn to the Albino Rats

Cage 9: IV + L.R + AZI + Zn + Sn

Albino rats	Weight	Volume	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1 tail mark	137g(f)	0.34ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
2 tail mark	146g(m)	0.37ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√		
3 tail mark	138g(f)	0.35ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
4 tail mark	160g(m)	0.40ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
5 tail mark	203g(m)	0.50ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√		
6 tail mark	138g(m)	0.35ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

*2 tail marked albino rat died on the 27th of November, 2023 and was not replaced.

*5 tail marked albino rat died on the 27th of November, 2023 and was not replaced.

The daily administration of Water and Albino Rat Feed Daily Drug Administration (28 Days)

Cage 10: Water + Albino Rat Feed

Albino rats	Weight	Volume	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1 tailmark	144g(f)	0.36ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
2 tailmark	186g(f)	0.46ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
3 tailmark	178g(f)	0.45ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
4 tailmark	154g(f)	0.39ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
5 tailmark	161g(m)	0.40ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
6 tailmark	160g(m)	0.40ml	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

Weight measured before and after CQ drug administration

S/N	Weight(grams) Before Drug Administration	Weight(grams) After Drug Administration
1	166 g	188 g
2	173 g	208 g
3	196 g	246 g
4	136 g	222 g
5	186 g	178 g
6	148 g	187 g

Weight measured before and after HCQ drug administration

S/N	Weight(grams) Before Drug Administration	Weight(grams) After Drug Administration
1	192 g	218 g
2	161 g	201 g
3	165 g	177 g
4	140 g	170 g
5	128 g	169 g
6	160 g	193 g

Weight measured before and after IV drug administration

S/N	Weight(grams) Before Drug Administration	Weight(grams) After Drug Administration
1	171 g	225 g
2	192 g	-
3	154 g	195 g
4	155 g	209 g
5	164 g	238 g
6	132 g	169 g

Weight measured before and after L/R drug administration

S/N	Weight(grams) Before Drug Administration	Weight(grams) After Drug Administration
1	122 g	186 g
2	110 g	151 g
3	152 g	193 g
4	163 g	209 g
5	126 g	176 g
6	133 g	175 g

Weight measured before and after IV drug administration

S/N	Weight(grams) Before Drug Administration	Weight(grams) After Drug Administration
1	171 g	225 g
2	192 g	-
3	154 g	195 g
4	155 g	209 g
5	164 g	238 g
6	132 g	169 g

Weight measured before and after L/R drug administration

S/N	Weight(grams) Before Drug Administration	Weight(grams) After Drug Administration
1	122 g	186 g
2	110 g	151 g
3	152 g	193 g
4	163 g	209 g
5	126 g	176 g
6	133 g	175 g

Weight measured before and after (AZI) drug administration

S/N	Weight(grams) Before Drug Administration	Weight(grams) After Drug Administration
1	188 g	156 g
2	153 g	225 g
3	184 g	262 g
4	125 g	197 g
5	144 g	178 g
6	127 g	171 g

Weight measured before and after Zn and Sn drug administration

S/N	Weight(grams) Before Drug Administration	Weight(grams) After Drug Administration
1	170 g	229 g
2	143 g	181 g
3	144 g	177 g
4	134 g	186 g
5	145 g	208 g
6	125 g	184 g

Weight measured before and after CQ, IV, L.R, AZI, Zn, and Se drug administration

S/N	Weight(grams) Before Drug Administration	Weight (grams) After Drug Administration
1	108 g	160 g
2	131 g	178 g
3	117 g	-
4	149 g	-
5	170 g	239 g
6	130 g	-

Weight measured before and after HCQ + AZI + L.R + IV + Zn + Se drug administration

S/N	Weight(grams) Before Drug Administration	Weight(grams) After Drug Administration
1	153 g	188 g
2	164 g	148 g
3	178 g	237 g
4	191 g	179 g
5	159 g	-
6	166 g	201 g

Weight measured before and after IV, L.R, AZI, Zn, and Sn drug administration

S/N	Weight(grams) Before Drug Administration	Weight(grams) After Drug Administration
1	137 g	210 g
2	146 g	-
3	138 g	180 g
4	160 g	193 g
5	203 g	-
6	138 g	192 g

ESTIMATION OF SERUM TOTAL CHOLESTEROL LEVEL BY ENZYMATIC END POINT METHOD

REAGENT COMPOSITION:

Pipes buffer	80 mmol/L, pH 6.8
4-aminoantipyrine	0.25 mmol/L
Phenol	6 mmol/L
Peroxidase	$\geq$ 0.5 U/mL
(E.C. 1.1.1.1.7, Horseradish + 25^0C)	
Cholesterolesterase	$\geq$ 0.15U/mL
(E.C.1.1.3.6. Nocardia, 37^0C)	$\geq$ 0.10 U/mL

ESTIMATION OF SERUM TRIGLYCERIDES (TRIGS) BY GPO-PAP METHOD

Reagent Composition:

Pipes buffer	40mmol/L, PH 7.6
4-chlorophenol	5.5 mmol/L
Magnesium ions	17.5mmol/L
4-aminophenazone	0.5mmol/L
ATP	1.0mmol/L
Lipases	$\geq$ 150 U/mL
Glycerol-Kinase	$\geq$0.4 U/mL
Glycerol-3-phosphate oxidase	$\geq$ 1.5 U/mL
Glycerol-3-phosphate oxidase	$\geq$1.5 U/mL
Peroxidase	$\geq$ 0.5 U/mL

ESTIMATION OF HIGH DENSITY LIPOPROTEIN (HDL-c) BY ENZYMATIC END POINT METHOD

Reagent Composition:

Contents	Concentrations in the test
R1. Posphotungstic Acid	0.55mmol/L
Magnesium Chloride	25mmol/L

SPSS OUTPUT FOR STATISTICAL ANALYSIS OF RESULTS

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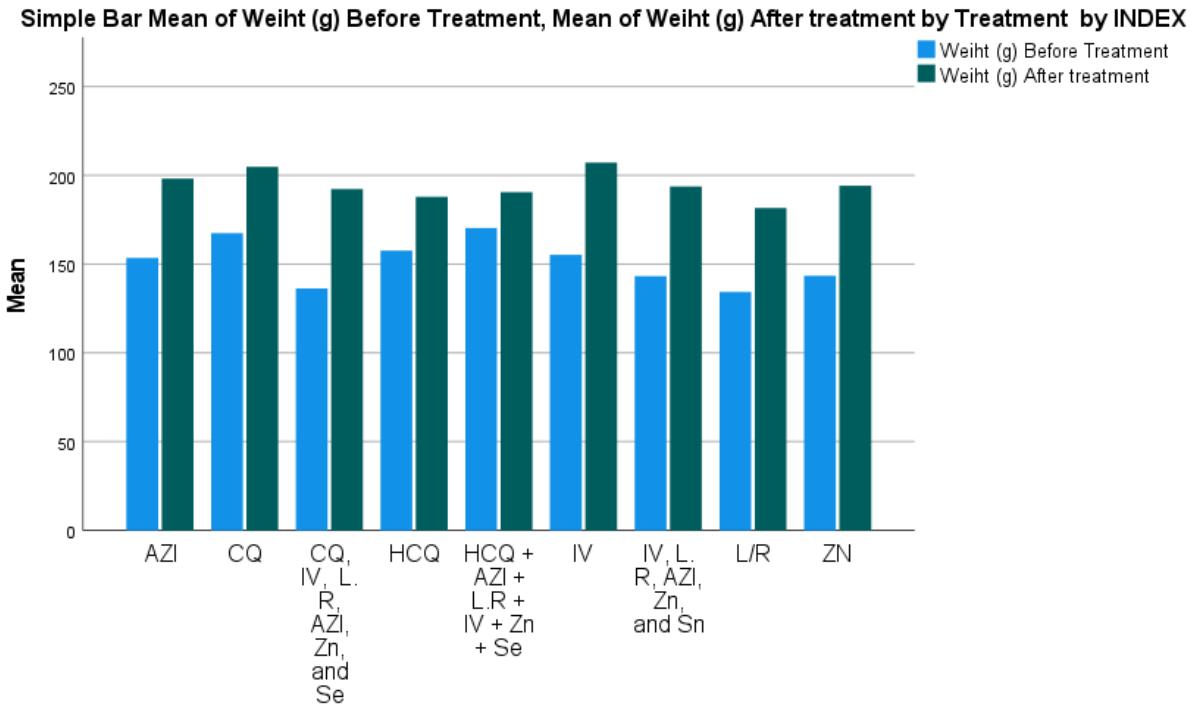

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BeforeHCQAZIL.RIVZnSeDruAdministration
BeforeIVL.RAZIZnandSnDruAdministration WITH AfterCQDruAdministration
AfterHCQDruAdministration
AfterIVDruAdministration AfterLRDruAdministration
AfterAZIDruAdministration
AfterZnDruAdministration AfterCQIVL.RAZIZnandSeDruAdministration
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T-Test

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Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	BeforeCQ Dru Administration	167.50	6	22.625	9.237
	After CQ Dru Administration	204.83	6	25.771	10.521
Pair 2	Before HCQ Dru Administration	157.67	6	22.097	9.021
	After HCQ Dru Administration	188.00	6	19.494	7.958
Pair 3	Before IV Dru Administration	155.20	5	14.721	6.583
	After IV Dru Administration	207.20	5	26.818	11.993
Pair 4	Before L/R Dru Administration	134.33	6	19.745	8.061
	After L/R Dru Administration	181.67	6	19.552	7.982
Pair 5	Before AZI Dru Administration	153.50	6	27.297	11.144
	After AZI Dru Administration	198.17	6	39.291	16.040
Pair 6	Before Zn Dru Administration	143.50	6	15.083	6.158
	After Zn Dru Administration	194.17	6	20.213	8.252
Pair 7	Before CQ, IV, L.R, AZI, Zn, and Se Dru Administration	136.33	3	31.342	18.095
	After CQ, IV, L.R, AZI, Zn, and Se Dru Administration	192.33	3	41.405	23.905
Pair 8	Before HCQ + AZI + L.R + IV + Zn + Se Dru Administration	170.40	5	14.536	6.501
	After HCQ + AZI + L.R + IV + Zn + Se Dru Administration	190.60	5	32.470	14.521
Pair 9	Before IV, L.R, AZI, Zn, and Sn Dru Administration	143.25	4	11.177	5.588
	After IV, L.R, AZI, Zn, and Sn Dru Administration	193.75	4	12.339	6.169

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	BeforeCQ Dru Administration & After CQ Dru Administration	6	.181	.732
Pair 2	Before HCQ Dru Administration & After HCQ Dru Administration	6	.876	.022
Pair 3	Before IV Dru Administration & After IV Dru Administration	5	.920	.027
Pair 4	Before L/R Dru Administration & After L/R Dru Administration	6	.898	.015
Pair 5	Before AZI Dru Administration & After AZI Dru Administration	6	.265	.611
Pair 6	Before Zn Dru Administration & After Zn Dru Administration	6	.792	.060
Pair 7	Before CQ, IV, L.R, AZI, Zn, and Se Dru Administration & After CQ, IV, L.R, AZI, Zn, and Se Dru Administration	3	.988	.100
Pair 8	Before HCQ + AZI + L.R + IV + Zn + Se Dru Administration & After HCQ + AZI + L.R + IV + Zn + Se Dru Administration	5	.204	.742
Pair 9	Before IV, L.R, AZI, Zn, and Sn Dru Administration & After IV, L.R, AZI, Zn, and Sn Dru Administration	4	-.079	.921

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	BeforeCQ Dru Administration - After CQ Dru Administration	-37.333	31.072	12.685	-69.941	-4.725	-2.943	5	.032
Pair 2	Before HCQ Dru Administration - After HCQ Dru Administration	-30.333	10.671	4.356	-41.532	-19.135	-6.963	5	.001
Pair 3	Before IV Dru Administration - After IV Dru Administration	-52.000	14.474	6.473	-69.972	-34.028	-8.033	4	.001
Pair 4	Before L/R Dru Administration - After L/R Dru Administration	-47.333	8.892	3.630	-56.665	-38.002	-13.039	5	.000
Pair 5	Before AZI Dru Administration - After AZI Dru Administration	-44.667	41.466	16.929	-88.183	-1.150	-2.639	5	.046

Pair 6	Before Zn Dru Administration - After Zn Dru Administration	-50.667	12.372	5.051	-63.650	-37.683	-10.031	5	.000
Pair 7	Before CQ, IV, L.R, AZI, Zn, and Se Dru Administration - After CQ, IV, L.R, AZI, Zn, and Se Dru Administration	-56.000	11.533	6.658	-84.648	-27.352	-8.411	2	.014
Pair 8	Before HCQ + AZI + L.R + IV + Zn + Se Dru Administration - After HCQ + AZI + L.R + IV + Zn + Se Dru Administration	-20.200	32.752	14.647	-60.867	20.467	-1.379	4	.240
Pair 9	Before IV, L.R, AZI, Zn, and Sn Dru Administration - After IV, L.R, AZI, Zn, and Sn Dru Administration	-50.500	17.292	8.646	-78.015	-22.985	-5.841	3	.010

Paired Samples Effect Sizes

			Standardizer[a]	Point Estimate	95% Confidence Interval Lower	Upper
Pair 1	BeforeCQ Dru Administration - After CQ Dru Administration	Cohen's d	31.072	-1.202	-2.250	-.093
		Hedges' correction	33.673	-1.109	-2.076	-.086
Pair 2	Before HCQ Dru Administration - After HCQ Dru Administration	Cohen's d	10.671	-2.843	-4.710	-.947
		Hedges' correction	11.564	-2.623	-4.346	-.874
Pair 3	Before IV Dru Administration - After IV Dru Administration	Cohen's d	14.474	-3.593	-6.129	-1.057
		Hedges' correction	16.034	-3.243	-5.533	-.954
Pair 4	Before L/R Dru Administration - After L/R Dru Administration	Cohen's d	8.892	-5.323	-8.613	-2.051
		Hedges' correction	9.636	-4.912	-7.948	-1.893
Pair 5	Before AZI Dru Administration - After AZI Dru Administration	Cohen's d	41.466	-1.077	-2.077	-.017
		Hedges' correction	44.938	-.994	-1.917	-.016
Pair 6	Before Zn Dru Administration - After Zn Dru Administration	Cohen's d	12.372	-4.095	-6.671	-1.517
		Hedges' correction	13.408	-3.779	-6.156	-1.400
Pair 7	Before CQ, IV, L.R, AZI, Zn, and Se Dru Administration - After CQ, IV, L.R, AZI, Zn, and Se Dru Administration	Cohen's d	11.533	-4.856	-9.439	-.552
		Hedges' correction	14.454	-3.874	-7.532	-.440

Pair 8	Before HCQ + AZI + L.R + IV + Zn + Se Dru Administration - After HCQ + AZI + L.R + IV + Zn + Se Dru Administration	Cohen's d	32.752	-.617	-1.556	.382
		Hedges' correction	36.282	-.557	-1.404	.344
Pair 9	Before IV, L.R, AZI, Zn, and Sn Dru Administration - After IV, L.R, AZI, Zn, and Sn Dru Administration	Cohen's d	17.292	-2.920	-5.329	-.513
		Hedges' correction	19.907	-2.537	-4.629	-.445

a. The denominator used in estimating the effect sizes.

Cohen's d uses the sample standard deviation of the mean difference.

Hedges' correction uses the sample standard deviation of the mean difference, plus a correction factor.

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Oneway

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Choloroquine                             2  Choloroquine
control                                  3  control
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HCQ + AZI + L.R + IV + Zn + Se           5  HCQ + AZI + L.R + IV + Zn + Se
Hydroxylcholoroquine                     6  Hydroxylcholoroquine
IV + L.R + AZI + Zn + Sn                 7  IV + L.R + AZI + Zn + Sn
Ivermectin                               8  Ivermectin
Lopinavir/Retinavir                      9  Lopinavir/Retinavir
Zinc/selenium                           10  Zinc/selenium


ONEWAY AIP BY treatment
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Oneway

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ETHICS COMMITTEE
FACULTY OF PHARMACY
UNIVERSITY OF BENIN, BENIN CITY, NIGERIA

P.M.B 115-
Telephone: + 2348033449913 +2348096351155
E-mail: omog20@yahoo.com ; omog@uniben.edu

5 August 202

Our Ref: EC/FP/020/13

Agnanowa Sylvester, PhD
Department of Pharmacology and Toxicology
Faculty of Pharmacy
University of Benin
Benin City
Nigeria

Dear Dr. Aghahowa.

RE – APPLICATION FOR ETHICAL APPROVAL FOR THE REVALIDATION OF THE EFFICACY AND TOXIC POTENTIAL OF DRUGS FOR THE TREATMENT OF COVID-19 INFECTION.

Your application for ethical approval of your proposal for the revalidation of the efficacy and toxic potential of drug molecules recommended for the treatment of COVID-19 infection has been reviewed by the Ethics Committee and you are hereby granted approval for the study.

Note that you are to adhere strictly to the methods described in your proposal.a Any need for protocol variation should be submitted to the Committee for further consideration.

Yours faithfully,

Prof. E. K. I. Omogbai
Chairman, Ethics Committee