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Evaluation of the Impact of Repurposed Covid-19 Medications on some Biochemical Markers in Albino Wistar Rats

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CERTIFICATION

This is to certify that this work was carried out by GRACE ELEOJO OBASUYI with matriculation number PG/BMS2110256 of the Department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin, Benin-City, under the supervision of Prof. H.B. Osadolor

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DEDICATION

This project work is dedicated to the ALMIGHTY GOD for His enabling strength which He bestowed on me to complete this research work and also to my husband, Dr. Michael Obasuyi and my lovely children Osasenaga, Evangel, Elijah, Osaenoma, and Gloria.

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ABSTRACT

The COVID-19 pandemic necessitated the rapid development and use of therapeutic drugs to combat the disease. However, concerns exist regarding the potential adverse effects of these drugs on metabolic processes like glucose homeostasis, renal function and bilirubin levels. This study aimed to evaluate the impact of chloroquine, hydroxychloroquine, ivermectin, azith romycin, lopinavir & ritonavir, zinc & selenium, which are recommended COVID-19 drugs, on glucose metabolism, renal function (urea and creatinine levels) and bilirubin levels in Wistar rat model. About sixty (60) Wistar rats were randomly assigned to 9 treatment test groups and a control group (a total of 10 groups). The drugs were administered orally at clinically relevant doses for one month. Blood glucose, urea, creatinine and bilirubin assay was performed to assess glucose metabolism using the oxidase-peroxidase method, bilirubin by Evelyn and Malloy's method, urea and creatinine levels using urease berthe lot's and alkaline picrate method respectively. Data obtained was analyzed by the Statistical Package for Social Sciences (SPSS) software. The results showed that the group treated with hydroxychloroquine + azith romycin + lopinavir/ritonavir + ivermectin + zinc + selenium demonstrated a markedly elevated mean glucose level of 103.80 mg/dL (P = 0.001) compared to the control (83.83mg/dL), indicating a statistically significant impact on glucose metabolism. Analysis revealed no significant difference in urea and creatinine levels ($p>0.05$) among the different groups as p values were = 0.109 and 0.848 respectively. The hydroxychloroquine + azith romycin + lopinavir/ritonavir + ivermectin + zinc + selenium group showed markedly elevated glucose levels. The direct bilirubin of experimental animals across most treated groups was significantly elevated ($p<0.05$). Results also showed that total bilirubin was significantly higher ($p<0.05$) in animals treated with ivermectin (0.93±0.10) and Lopinavir-ritonavir (0.92±0.06) when compared to control (0.47±0.07) . In conclusion, patients who are being administered this drug combination (hydroxychloroquine + azith romycin + lopinavir/ritonavir + ivermectin + zinc + selenium) are at risk of developing diabetes mellitus and also further worsening the condition of diabetic patients. Also administration of these drugs may induce liver dysfunction, hyperbilirubinemia, drug-induced liver injury, drug-induced hepatitis and consequently jaundice. It is recommended to avoid the concurrent use of this specific drug combination unless the potential benefits outweigh the risks of hyperglycemia, the administration of these drugs adversely affected the synthetic and excretory functions of the liver and regular assessment of liver function parameters necessary.

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CHAPTER ONE
INTRODUCTION

A. Background of the Study

The viruses that cause respiratory and interstitial infections, which can range from cold-like symptoms to severe respiratory failure, are called coronaviruses (CoVs). They are spherical, enclosed viruses with a positive sense single- stranded RNA genome (Cui et al., 2019; Pollard et al., 2020). Giovanetti et al., (2021) and Feh r and Perlman (2015) An outbreak of the new coronavirus SARS- CoV-2 occurred in Wuhan, China in 2019. The WHO declared the COVID-19 pandemic in February 2020 as a result of the disease's high contagiousness (Hassan etal., 2020; Lai et al., 2020). COVID-19 is caused by the SARS-virus type 2. There have been more than 6.7 million recorded deaths and over 664 million confirmed cases worldwide as of January 2023. Asymptomatic for two to fourteen days, COVID-19 is typically spread by respiratory droplets. As a pandemic, it threatened lives worldwide, led to lockdowns, paralyzed transportation systems, and spurred the repurposing of various drugs for clinical trials and treatment. The safety of these repurposed drugs for COVID-19 patients are however still in doubt as they may cause adverse effects which may include renal complications (Yarijani and Najaf i, 2021).

Chloroquine and hydroxychloroquine are antimalarial drugs that have been around for a while. After being developed by the Germans in 1939, chloroquine had supplanted quinacrine as the malaria treatment of choice by the end of World War II because it was safer and more effective (Devaux et al., 2020). The hydroxyl group that sets hydroxychloroquine apart from chloroquine and makes it safer was added in 1955. These medications not only contain antiviral and anti- inflammatory qualities, but also antimalarial ones. Acid vesicles are impacted, many enzymes are inhibited, and viral entrance into cells might be hindered when endocytosis is dependent on pH. Additionally, they prevent post-translational modifications and viral g lycosyl-transferases. These medications were tested for the treatment of COVID-19 due to the urgency of the pandemic and encouraging in vitro investigations (Sinha and Balayla, 2020).

The macrolide antibiotic azith romycin has extra antiviral and anti-inflammatory properties (Parnham etal., 2014), which may aid in the treatment of COVID-19. It may impede the spread of SARS-CoV-2 in several ways: 1) Azith romycin accumulates intracellularly and elevates pH, potentially compromising Golgi/lysosome function crucial for viral replication (Arabi et al., 2020); 2) Azith romycin shares similarities in size and chemistry with ganglioside GM1. Azith romycin has the potential to bind the SARS-CoV-2 spike protein and inhibit its interaction with gangliosides, hence preventing viral entrance, as this contact is necessary for the virus to infect host cells (Fantini et al., 2020). In order to potentially prevent SARS-CoV-2 infection and treat COVID-19 illness, azith romycin possesses both extracellular and intracellular mechanisms.

As an adjuvant for ritonavir, lopinavir (LPV) is an antiretroviral medication used to treat and prevent HIV infection. The HIV-1 protease enzyme, which is essential for viral replication, is inhibited by LPV, a peptidomimetic. LPV stops the cleavage of viral polyprotein precursors into mature, functional proteins that are required for viral replication by attaching to the catalytic site of this protease. Ritonavir, which is usually used in small doses with LPV, improves the pharmacokinetics of LPV by inhibiting the cytochrome P450 3A4 enzyme, which slows down LPV metabolism in the liver and increases LPV's therapeutic efficacy. Research demonstrating LPV/r's suppression of SARS-CoV-2's primary protease, albeit with less efficacy than HIV's protease due to structural differences, has also led to its repurposing for the treatment of COVID-19 (Costanzo etal., 2020).

Two crucial markers that are used to evaluate kidney function and identify kidney disease are creatinine and urea. Protein metabolism produces urea as a byproduct, and increased protein breakdown or decreased renal filtration can raise urea levels in the blood (BUN). On the other hand, things like food and dehydration can affect BUN. Conversely, serum creatinine levels in the blood are more specific for assessing kidney function since creatinine is a breakdown product of muscle creatine. Elevated serum creatinine levels usually suggest compromised kidney function or renal injury because creatinine is filtered out by the kidneys and its production is rather steady. Renal function is evaluated using serum creatinine and BUN levels as well as the estimated glomerular filtration rate (GFR) (Salazar, 2014). Chronic renal disease may be indicated by persistently high levels of these markers (Kamal, 2014).

One area of concern is the impact of COVID-19 drugs on glucose metabolism and body weight regulation. Maintaining normal glucose levels and healthy body weight is crucial for overall health and well-being, as dysregulation in these parameters can lead to various metabolic disorders, including diabetes, obesity, and cardiovascular diseases (Bays et al., 2021). COVID-19 itself has been associated with an increased risk of developing diabetes and metabolic complications, further highlighting the importance of understanding the potential adverse effects of COVID-19 drugs on glucose homeostasis and weight regulation (Rubino etal., 2020).

Another COVID-19 drug that has gained widespread attention is dexamethasone, a potent corticosteroid with anti-inflammatory and immunomodulatory properties (Okubo et al., 2019). Dexamethasone has been shown to reduce mortality in severe COVID-19 cases requiring respiratory support. However, corticosteroids are known to have metabolic side effects, including insulin resistance, hyperglycemia, and weight gain (Sohail et al., 2017). Therefore, it is essential to investigate the

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potential adverse effects of dexamethasone and other corticosteroids used in the treatment of COVID-19 on glucose levels and body weight regulation

COVID-19 pandemic has necessitated the rapid development and deployment of effective drug treatments. However, it is essential to thoroughly investigate the potential adverse effects of these drugs, particularly on metabolic processes such as glucose metabolism.

However, significant concerns have emerged regarding the potential liver toxicity of these drugs being explored for COVID-19 treatment. This necessitates a thorough examination of their harmful and damaging effects on the liver's crucial protein synthesis functions. The liver plays a vital role in producing albumin, a protein abundantly present in the bloodstream, as well as conjugating bilirubin for further metabolism. Albumin is the most plentiful circulating protein in human plasma, accounting for approximately half of the total plasma protein content, ranging from 3.5 to 5 g/dl in healthy individuals. Hepatocytes, the liver's specialized cells, continuously synthesize albumin, which is rapidly secreted into the bloodstream at a rate of about 10 to 15 grams per day. The liver stores minimal amounts of albumin, with the majority being promptly released into circulation. In the human body, serum albumin serves as a significant regulator of plasma oncotic pressure and acts as a transporter for various endogenous and exogenous substances, including drugs. Routine blood tests can measure serum albumin levels in clinical laboratory settings, providing valuable diagnostic information. As researchers evaluate potential COVID-19 therapies, it is crucial to comprehensively assess their impact on the liver's essential albumin production and other crucial protein synthesis functions to ensure patient safety and well- being. As a laboratory parameter, serum albumin can furnish clinicians with invaluable insights into patients' hepatic functionality or their capacity to biosynthesize proteins and factors that are indispensable for maintaining total body homeostasis. Observations have indicated that abnormalities in liver function tests, which encompass aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, total protein, bilirubin, and other markers, generally resolve upon the remission of COVID-19 or the discontinuation of hepatotoxic drugs (Yuwen et al., 2017). These aberrations in liver function test results are associated with drug-induced liver injury (DILI), which can be attributed to the excessive utilization of antimalarial, antiviral, and antimicrobial agents. Studies have documented varying degrees of hepatic injury in COVID-19 patients, individuals with severe illness exhibit significantly elevated levels of hepatic dysfunction, which is correlated with poor clinical outcomes. Elevated bilirubin concentrations and diminished albumin synthesis serve as indicators of hepatic dysfunction (Yuwen et al., 2017).

B. Statement of Problem

COVID-19 repurposed medications such as hydroxy chloroquine, chloroquine, Ivermectin, Azith romycin, Lopinavir/Rotanavir, Zinc and Selenium have been used to test COVID-19 infections but the safety of these drugs are not well known. This study aims to know how safe these repurposed medications are on the kidney's liver and glucose metabolism

C. Justification

According to Salazar (2014), urea and creatinine are markers of renal impairment and function. Increased blood levels of these compounds may be a sign of acute or chronic kidney injury, which can have detrimental effects on a patient's quality of life and ability to survive. Azith romycin and other repurposed medications, including hydroxychloroquine, chloroquine, and lopinavir/ritonavir, have been linked in earlier research to nephrotoxicity, or kidney toxicity, in COVID-19 patients (Yarijani and Najaf i, 2021; Edelstein et al., 2020). The mechanisms of nephrotoxicity are unclear, and the available data is sparse and contradictory.

Dysregulation of glucose levels and unhealthy changes in body weight can lead to serious metabolic disorders, including diabetes, obesity, and cardiovascular complications. These conditions not only pose significant health risks to patients but may also exacerbate the severity of COVID-19 and impede recovery. Furthermore, COVID-19 itself has been associated with an increased risk of developing metabolic complications, further underscoring the importance of understanding the potential adverse effects of COVID-19 drugs on glucose homeostasis and weight regulation. The disadvantage in the use is that the adverse effects of these drugs have not been established enough, therefore this study aims to elaborate the adverse effects of these drugs with particular interest to liver function.

D. Aim of the Study

The aim of this study is to evaluate the impact of repurposed Covid-19medications on some renal and liver biomarkers in Wistar rats.

E. Objectives of the Study

- To evaluate the impact of COVID-19 drug treatment on fasting blood glucose levels in albino Wistar rats.
- To assess the basal and post-treatment concentrations of urea and creatinine in the serum of test and control rats that had received any of the pharmaceuticals.
- To estimate the harmful outcomes of the drugs on total bilirubin and direct bilirubin levels.

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F. Research Question

- What is the impact of COVID-19 drug treatment on fasting blood glucose levels in albino Wistar rats?
- What is the basal and post-treatment concentrations of urea and creatinine differ between test and control rats administered with pharmaceuticals?
- What are the harmful effects of the drugs on total bilirubin and direct bilirubin levels in albino Wistar rats?

G. Research Significance

Provide new and comprehensive data on the effects of repurposed drugs on kidney function and damage in albino rats, liver function and dysfunction in albino Wistar rats, and glucose metabolism which can serve as a basis for further research and clinical trials in humans.

Assist in clarifying the fundamental processes behind the nephrotoxicity or nephroprotection of repurposed medications and hepatoprotection or hepatotoxicity of repurposed drugs as this may result in the creation of freshtactics and treatments to stop or cure kidney damage and liver damage in COVID-19 Patients.

Positive impact on the health of patients with covid-19, especially those with diabetes mellitus or comorbidities, by providing them with safer and more effective treatments.

H. Hypothesis

➢ *Null Hypothesis*
Using repurposed drugs on Covid-19 patients increase the risk of having diabeticmellitus.

- *Alternative Hypothesis*
Using repurposed drugs on Covid-19 patients decrease the risk of having diabetic mellitus.

➢ *Null Hypothesis*
Using repurposed drugs has adverse effects on the kidney in COVID-19 patients

- *Alternative Hypothesis*
Using repurposed drugs has no adverse effect on the kidney in COVID-19 patients.

➢ *Null Hypothesis*
Using repurposed drugs has adverse effects on the liver in COVID-19 patients.

- *Alternative Hypothesis*
Using repurposed drugs has no adverse effect on the liver in COVID-19 patients.

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CHAPTER TWO
LITERATURE REVIEW

A. Brief Histrory of Covid-19

COVID-19, or Coronavirus Disease 2019, is a highly contagious respiratory illness caused by the SARS-CoV-2 virus, which belongs to the coronavirus family. This novel virus was first identified in Wuhan, China, in late 2019 and quickly spread globally, leading to a pandemic that has profoundly impacted public health, economies, and societies worldwide.

The COVID-19 pandemic has profoundly impacted the world, disrupting lives, economies, and societies. This unprecedented global crisis has highlighted the importance of understanding the causative agent, transmission dynamics, and effective preventive measures. This write-up aims to provide a comprehensive overview of the COVID-19 virus, its causative organism, mode of transmission, prevention strategies and the various strains that have emerged during the pandemic.

B. The Causative Organism: SARS-CoV-2

COVID-19 is caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), a novel strain of the coronavirus family (Coronaviridae). Coronaviruses are enveloped, single-stranded RNA viruses that can infect humans and various animal species (Zhu et. al. 2020). SARS-CoV-2 is a betacoronavirus, closely related to the viruses that caused the severe acute Respiratory syndrome (SARS) and Middle East Respiratory Syndrome (MERS)outbreaks (Andersen et. al. 2020).

C. Virology and Structure

SARS-CoV-2 is a spherical virus with a diameter ranging from 60 to 140 nanometers (Zhu et. al. 2020). Its genome consists of a single-stranded, positive- sense RNA molecule of approximately 30,000 nucleotides, encoding for various structural and non-structural proteins (Walls et. al. 2020). The virus's surface is adorned with spike (S) proteins, which play a crucial role in binding to host cell receptors and facilitating viral entry (Shang et. al. 2020).

D. Mode of Transmission

SARS-CoV-2 is primarily transmitted through respiratory droplets expelled when an infected person coughs, sneezes, or talks (WHO, 2020a). These droplets can land in the mouths, noses, or eyes of nearby individuals, leading to infection. Airborne transmission can also occur in specific settings, such as poorly ventilated indoor spaces (Greenhalg h et. al. 2021). Additionally, the virus can be transmitted through contact with contaminated surfaces or objects, although this mode of transmission is less common (CDC, 2021a).

E. Symptoms and Clinical Manifestation

COVID-19 can cause a range of symptoms, from mild to severe. The most common symptoms include fever, cough, fatigue, and shortness of breath (CDC, 2021b). Other reported symptoms may include muscle pain, headache, sore throat, and loss of taste or smell (WHO, 2020b). In severe cases, COVID-19 can lead to pneumonia, acute respiratory distress syndrome (ARDS), and multi-organ failure, which can be life-threatening, particularly for individuals with underlying health conditions or compromised immune systems (Huang et. al. 2020).

F. Prevention and Recommendations

Effective prevention strategies are crucial in mitigating the spread of COVID-19 and reducing the burden on healthcare systems. The WHO and various national and international health organizations have issued guidelines and recommendations for preventing the transmission of SARS-CoV-2.

G. Epidemiology and Global Spread

COVID-19 has exhibited an unprecedented global spread, with cases reported in virtually every country and territory worldwide. As of August 2023, the World Health Organization (WHO) has reported over 700 million confirmed cases and over 6.8 million deaths globally (WHO, 2023). The pandemic has had a significant impact on healthcare systems, economies, and social structures worldwide.

H. Regulatory Bodies and Response

The COVID-19 pandemic has prompted a coordinated global response from various regulatory bodies and organizations. The WHO has played a pivotal role in monitoring the spread of the virus, issuing guidelines, and coordinating international efforts to combat the pandemic bodies and organizations. The WHO has played a pivotal role in monitoring the spread of the virus, issuing guidelines, and coordinating international efforts to combat the pandemic. The organization declared COVID-19 a Public Health Emergency of International Concern on January 30, 2020, and a pandemic on March 11, 2020 (WHO, 2020a).

National and regional regulatory bodies, such as the Centers for Disease Control and Prevention (CDC) in the United States, the European Centre for Disease Prevention and Control (ECDC), and national health ministries, have also been instrumental in implementing public health measures, conducting surveillance and providing guidance to healthcare professionals and the general public.

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I. Vaccine Development and Distribution

One of the most significant achievements in the fight against COVID-19 has been the rapid development and deployment of several highly effective vaccines. Regulatory bodies, such as the U.S. Food and Drug Administration (FDA), the European Medicines Agency (EMA), and the WHO, have played crucial roles in evaluating the safety and efficacy of these vaccines and facilitating their distribution worldwide (CDC, 2021c).

As of August 2023, several vaccines have received emergency use authorization or full approval from various regulatory bodies, including those developed by Pfizer-BioNTech, Moderna, AstraZeneca, Johnson & Johnson, and others. These vaccines have been instrumental in reducing the severity of the pandemic and saving countless lives.

J. Personal Protective Measures

- Hand Hygiene: Frequent handwashing with soap and water or the use of alcohol-based hand sanitizers is recommended to prevent the spread of the virus (WHO, 2020c).
- Respiratory Etiquette: Covering coughs and sneezes with a tissue or the elbow, and appropriate disposal of used tissues, is essential to prevent the spread of respiratory droplets (CDC, 2021c).
- Physical Distancing: Maintaining a distance of at least 1 meter (3 feet) from others, especially in crowded or poorly ventilated areas, can reduce the risk of transmission (WHO, 2020d).
- Face Masks: The use of face masks or coverings, particularly in public settings where physical distancing is difficult to maintain, can help prevent the spread of respiratory droplets (CDC, 2021d).

K. Environmental and Community Measures

- Ventilation: Ensuring adequate ventilation in indoor spaces by opening windows, using air purifiers, or improving ventilation systems can help reduce airborne transmission (CDC, 2021e).
- Cleaning and Disinfection: Regular cleaning and disinfection of frequently touched surfaces and objects can help minimize the risk of transmission through contact (WHO, 2020e).
- Vaccination: COVID-19 vaccines have been developed and made available to the public, providing a crucial tool in preventing severe illness and reducing the burden on healthcare systems (CDC, 2021f).
- Testing and Contact Tracing: Widespread testing and effective contact tracing can help identify and isolate infected individuals, thereby slowing the spread of the virus (WHO, 2020f).

L. Types of Corona Virus Strains

Throughout the COVID-19 pandemic, various strains of SARS-CoV-2 have emerged due to mutations in the viral genome. These strains, or variants, can differ in terms of transmissibil ity, severity, and potential to evade immune responses. Some notable variants include:

- Alpha (B.1.1.7) Variant: First identified in the United Kingdom in late 2020, this variant was associated with increased transmissibil ity (Volz et. al. 2021).
- Beta (B.1.351) Variant: Discovered in South Africa, this variant showed potential for increased resistance to certain COVID-19 vaccines and monoclonal antibody treatments (Tegally et. al. 2021).
- Gamma (P.1) Variant: Originating in Brazil, this variant exhibited increased transmissibil ity and the potential for reinfection (Faria et. al. 2021).
- Delta (B.1.617.2) Variant: First identified in India, the Delta variant was highly transmissible and became the dominant strain globally in 2021 (Planas et. al. 2021).
- Omicron (B.1.1.529) Variant: Emerged in late 2021, the Omicron variant had numerous mutations and exhibited increased transmissibil ity and potential for immune evasion (Callaway, 2021).

M. Ongoing Survei Ance and Research

As the COVID-19 pandemic continues to evolve, ongoing surveillance and research efforts are crucial to monitor the emergence of new variants, understand their characteristics, and develop effective countermeasures. Collaborative efforts among scientists, public health agencies and regulatory bodies are essential to stay ahead of the virus and mitigate its impact on global health.

N. Ongoing Challenges and Future Outlook

Despite the remarkable progress made in combating COVID-19, several challenges remain. The emergence of new variants, such as the Delta and Omicron variants, has raised concerns about the potential for increased transmissibil ity, immune evasion, and reduced vaccine effectiveness (WHO, 2021). Ongoing surveillance, research, and vaccine development efforts are crucial to address these evolving challenges.

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Additionally, issues related to vaccine hesitancy, equitable distribution, and access to healthcare resources continue to pose obstacles, particularly in low- and middle-income countries. Addressing these challenges will require concerted efforts from regulatory bodies, governments, healthcare organizations, and the global community.

As the COVID-19 pandemic continues to evolve, regulatory bodies will play a crucial role in monitoring the situation, issuing guidelines, and coordinating efforts to mitigate the impact of the virus and protect public health. Ongoing research, international collaboration, and a commitment to evidence-based decision-making will be essential in navigating the path forward. (WHO, 2021)

O. Pharmacological interventions and Supplements in the Management of COVID-19

The unprecedented global COVID-19 pandemic has prompted extensive research efforts to identify effective p harmacological interventions and potential therapeutic agents. While several drugs and supplements were initially considered for their potential benefits in treating COVID-19, their clinical efficacy and safety have been extensively debated and evaluated. This write-up examines the following agents: chloroquine, hydroxychloroquine, ivermectin, azith romycin, lopinavir, and ritonavir, and the supplements zinc and selenium.

➢ *Chloroquine and Hydroxychloroquine*

- *Class and Mechanism of Action*

Chloroquine and hydroxychloroquine are antimalarial drugs belonging to the quinoline class of medications. They are also used in the treatment of autoimmune disorders such as rheumatoid arthritis and lupus erythematosus due to their immunomodulatory properties (Schrezenmeier and Dörner, 2020).

The proposed mechanism of action against SARS-CoV-2 involves increasing the endosomal pH, which interferes with viral entry and replication (Devaux et. al. 2020).

- Pharmacokinetics

Both drugs are well-absorbed after oral administration and have a large volume of distribution, allowing them to accumulate in tissues, including the lungs (Schrezenmeier and Dörner, 2020). Hydroxychloroquine has a longer half-life and lower risk of toxicity compared to chloroquine (Ramírez et. al. 2021).

- Adverse Effects

The use of chloroquine and hydroxychloroquine is associated with several potential adverse effects, including gastrointestinal disturbances, retinopathy, cardiac arrhythmias (prolonged QT interval), and hypoglycemia (Ramírez et. al. 2021; Schrezenmeier and Dörner, 2020). Careful monitoring and consideration of risk factors are necessary when using these drugs.

➢ *Ivermectin*

- *Class and Mechanism of Action*

Ivermectin is an antiparasitic drug belonging to the anti-helmintics class. It is primarily used for the treatment of parasitic infections, such as onchocerciasis and strongyloidiasis (Crump and Omura, 2011). The proposed mechanism of action against SARS-CoV-2 involves inhibiting the viral replication through several pathways, including blocking the importin alpha/beta-mediated nuclear transport and inhibiting the RNA he licase activity (Jermain et. al. 2020; Caly et. al. 2020).

- *Pharmacokinetics*

Ivermectin is well-absorbed after oral administration and has a high volume of distribution, allowing it to reach various tissues (González Canga et. al. 2008). However, its use in COVID-19 has been controversial due to the high doses required to achieve antiviral effects, which may lead to safety concerns (Navarro et. al. 2020).

- *Adverse Effects*

Ivermectin is generally well-tolerated at standard doses used for parasitic infections. However, potential adverse effects include gastrointestinal disturbances, dizziness, and neurological effects, particularly at higher doses (Navarro et. al. 2020; Crump and Omura, 2011).

➢ *Azith Romycin*

- *Class and Mechanism of Action*

Azith romycin is a macrolide antibiotic commonly used for the treatment of bacterial infections, such as respiratory tract infections, skin and soft tissue infections, and certain sexually transmitted diseases (Zuckerman, 2014). The proposed

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mechanism of action against SARS-CoV-2 involves its immunomodulatory and anti-inflammatory properties, as well as potential antiviral effects through interference with viral entry and replication (Arabi et. al. 2020).

- *Pharmacokinetics*

Azith romycin is well-absorbed after oral administration and has a large volume of distribution, allowing it to accumulate in tissues, including the Lungs (Zuckerman, 2014). Its' long half-life allows for once-daily dosing and extended tissue concentrations (Zuckerman, 2014).

- Abverse Effects

Common adverse effects of azith romycin include gastrointestinal disturbances, dizziness, and headache. Additionally, it may prolong the QT interval, increasing the risk of arrhythmias, especially when used in combination with other QT- prolonging drugs (Zuckerman, 2014; Arabi et. al. 2020).

➢ *Lopinavir and Ritonavir*

- *Class and Mechanism of Action*

Lopinavir and Ritonavir are protease inhibitors primarily used in combination for the treatment of HIV/AIDS (Hegazi et al., 2021). The proposed mechanism of action against SARS-CoV-2 involves inhibiting the viral protease, which is essential for viral replication (Hegazi et. al. 2021).

- *Pharmacokinetics*

Lopinavir has a low bioavailability when administered alone, but co- administration with Ritonavir; is a potent inhibitor of cytochrome P450 3A4 (CYP3A4), and increases its plasma concentrations significantly (Hegazi et. al. 2021). Both drugs have a high volume of distribution and are metabolized by CYP3A4 (Hegazi et. al. 2021).

- *Adverse Effects*

Common adverse effects of Lopinavir and Ritonavir include gastrointestinal disturbances, hyperlipidemia, and potential hepatotoxicity. Additionally, they may interact with other medications metabolized by CYP3A4, leading to increased or decreased drug concentrations (Hegazi et. al. 2021).

- *Zinc and Selenium*

Zinc and Selenium are essential micronutrients that play crucial roles in various biological processes, including immune function and antioxidant defense mechanisms (Ska lny et. al. 2020; Majeed et. al. 2022).

- *Role in COVID-19*

The proposed mechanisms by which Zinc and Selenium may be beneficial in COVID-19 include their immunomodulatory and anti-oxidant properties, as well as their potential to inhibit viral replication (Ska lny et. al. 2020; Majeed et. al. 2022). However, the clinical evidence for their efficacy in COVID-19 remains inconclusive, and further research is needed.

- *Pharmacokinetics*

Zinc and selenium are absorbed through the gastrointestinal tract, and their bioavailability can be influenced by various factors, such as dietary sources, interactions with other nutrients, and individual physiological factors (Ska lny et. al. 2020; Majeed et. al. 2022).

- Adverse Effects

High doses of Zinc and Selenium supplements can lead to adverse effects, including gastrointestinal disturbances, interference with the absorption of other essential minerals, and potential toxicity (Ska lny et. al. 2020; Majeed et. al. 2022). It is crucial to follow recommended dietary intake guidelines and consult healthcare professionals before supplementation.

P. Urea

Urea, having the chemical composition CO(NH2)2, is an organic compound that is colorless, odorless, and readily soluble. It serves as the main nitrogenous waste resulting from protein metabolism in mammals, including humans. The liver synthesizes urea through the urea cycle, also referred to as the ornithine cycle, and it is primarily eliminated from the body through the kidneys in urine (Kulkman et al., 2020).

The liver goes through a series of biochemical events known as the urea cycle, which produces urea from ammonia and carbon dioxide. The process begins with the enzyme carbamoyl phosphate synthetase I (CPS I), which aids in the transformation of ammonia into carbamoyl phosphate. Ornithine transcarbamylase (OTC) is the enzyme that catalyzes the subsequent reaction between carbamoyl phosphate and ornithine to create citrulline. With the aid of the enzyme argininosuccinate synthetase, citrulline enters the cytoplasm and combines with aspartate to produce argininosuccinate.

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Argininosuccinate lyase subsequently transforms argininosuccinate into arginine. The last phase involves the hydrolysis of arginine by the enzyme arginase, which yields urea and ornithine, which can reenter the cycle (Kulkman et al., 2020).

Upon hepatic synthesis, urea gains access to the circulatory system for transport to renal structures, which facilitates its elimination. In the kidneys, urea initially undergoes filtration in the glomeruli. Subsequently, it encounters processes of reabsorption and secretion as it traverses the renal tubules. Ultimately, the amount of urea excreted in renal outputs is influenced by various factors, including the flow rate of blood to the kidneys, the rate of glomerular filtration (GFR), and the functional competence of the renal tubules (Hirsch et al., 2020).

When evaluating renal function in clinical practice, urea is a crucial component. Accompanying other metrics like serum creatinine, serum urea levels —often measured as blood urea nitrogen, or BUN — are frequently employed as a kidney function indicator. As the kidneys are unable to efficiently filter and eliminate urea, elevated BUN levels may be a sign of compromised renal function (Kamal, 2014).

Conversely, lower BUN readings could indicate a decrease in protein consumption or an increase in renal clearance as a result of diuretic medication or volume expansion (Cheng et al., 2020; Salazar, 2014).

Dehydration, acute kidney injury (AKI), and chronic Kidney disease (CKD) are just a few of the renal disorders that require serum urea level testing to be diagnosed and tracked. According to Uch ino et al. (2012), decreased glomerular filtration and tubular reabsorption are reflected in increased BUN levels that frequently accompany a rapid loss in renal function in AKI. As kidney function declines over time in chronic kidney disease (CKD), higher BUN levels may be a sign of this progression (Pandya et al., 2016). BUN-to-creatinine ratio calculations are also made using creatinine levels and BUN levels, and the results can offer important information about the underlying cause of renal failure (Gowda etal., 2010).

Although blood urea nitrogen (BUN) levels are routinely measured in clinical settings, their interpretation has certain limitations. BUN levels can be influenced by factors unrelated to Kidney function, such as dietary protein intake, catabolic states, and gastrointestinal bleeding. Moreover, BUN readings may be affected by non-renal conditions like liver disease, heart failure, and dehydration, complicating the assessment of results (Brooke's and Power, 2022). As such, BUN levels should not be evaluated in isolation but rather in combination with other renal function tests and clinical findings to accurately gauge Kidney function and guide appropriate patient management decisions (Gowda et al.,2010).

Q. Creatinine

Creatinine is a waste byproduct that arises from the breakdown of creatine phosphate in muscle tissues (Kashani et al., 2020). Its production occurs at a relatively steady rate, and it is eliminated from the body by the kidneys. Creatinine is a nitrogen-containing compound with the chemical formula C4H7N3O and a molecular weight around 113.12 g/mol. In contrast to urea, another waste product, creatinine does not undergo significant reabsorption by the kidneys, rendering it a reliable indicator for evaluating kidney function.

The biosynthesis of creatinine primarily occurs in the muscles through the degradation of creatine phosphate, a molecule involved in the storage and transfer of energy in muscle cells. Creatine phosphate donates its phosphate group to adenosine diphosphate (ADP), converting it back into adenosine triphosphate (ATP) during periods of high energy demand, such as muscle contraction. This reaction results in the formation of creatinine as a byproduct (Kashani et al., 2020).

After its formation, creatinine travels through the bloodstream to the kidneys, where the renal glomeruli filter it from the blood into the urine. Unlike certain other waste substances, creatinine undergoes minimal reabsorption by the renal tubules. Hence, the rate at which creatinine is expelled in the urine correlates directly with the glomerular filtration rate (GFR), which indicates renal function (Nank ivell, 2001).

The measurement of serum creatinine levels is a fundamental component of assessing renal function in clinical practice (Salazar, 2014). Elevated levels of creatinine in the blood indicate impaired renal function, as the kidneys are unable to effectively filter and excrete creatinine. Conversely, decreased creatinine levels may indicate reduced muscle mass or decreased production due to muscle-wasting conditions (Carrero etal., 2016).

Serum creatinine levels are routinely measured alongside other markers like blood urea nitrogen (BUN) to assess kidney function. The ratio of BUN to creatinine can offer insights into the underlying cause of renal dysfunction. For instance, an elevated BUN-to-creatinine ratio may indicate prerenal causes of kidney injury, such as dehydration or reduced blood flow to the kidneys, whereas a low ratio could suggest an intrinsic renal pathology like acute tubular necrosis (Salazar, 2014; Esson and Sch rier, 2002).

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While creatinine is a widely used marker for renal function, it has certain limitations. For instance, serum creatinine levels may not accurately reflect renal function in individuals with altered muscle mass, such as the elderly or those with muscle-wasting diseases. Additionally, creatinine levels may not rise until there is a significant impairment of renal function, leading to a delayed diagnosis of kidney disease (Koyner, 2012).

Aside from evaluating serum creatinine levels, assessing creatinine clearance offers a more precise evaluation of GFR. Creatinine clearance is determined by measuring the rate at which creatinine is eliminated in the urine during a defined timeframe and comparing it to the serum creatinine level. This computation considers variables like age, sex, and body weight to provide a more refined estimation of renal function (Nank ivell, 2001).

R. COVID-19 and Acute Kidney Injury

Although the majority of COVID-19's effects are respiratory, acute kidney damage (AKI) is becoming more widely acknowledged as a frequent consequence that frequently manifests during hospital a?0zdmission. Although later research from the USA and Europe shows significantly higher rates of AKI, particularly in intensive care settings, with up to 45% of ICU patients requiring kidney replacement therapy (KRT), early reports from China suggested relatively low rates of kidney involvement (Battle et al., 2020; Cheng et al., 2020). According to Gupta et al. (2021), hospitalized COVID-19 patients with AKI have worse fatality rates than those without renal dysfunction. Although assessing long-term effects is difficult due to protracted hospitalizations and inadequate follow-up data, anecdotal accounts imply reduced renal recovery in survivors compared to other kinds of AKI (Cummings et al., 2020).

Differences in demographic comorbidities and AKI diagnosis and reporting techniques make it difficult to determine the exact epidemiology of COVID-19 AKI. Age, diabetes mellitus, and hypertension have all been repeatedly linked to increased risk of AKI in COVID-19 individuals. According to multiple studies (Carlson et al., 2021; Williamson et al., 2020), chronic kidney disease (CKD) is associated with worse COVID-19 results, making it a substantial risk factor for AKI. Complicate conditions such as diabetes, hypertension, and obesity frequently accompany chronic kidney disease (CKD) and are associated with a higher risk of COVID-19 death (Cheng et al., 2020).

Cantal uppi et al. (2020) suggest that decreasing nephron mass and renal functional reserve (RFR) may be the cause of the high mortality in older and comorbid patients. RFR and GFR declines may be a factor in the development of AKI, according to epidemiological research. As per Peng et al. (2020), a Wuhan study, there exists a correlation between inflammatory biomarkers, CKD, AKI, and advanced age. According to studies conducted in Spain and Italy, CKD and inflammatory markers were found to be significant predictors of higher AKI rates (Portolés et al., 2020; Russo et al., 2021). There are various sub-phenotypes of COVID-19 AKI despite the complex pathogenesis.

Many studies have indicated that repurposed medications raise the likelihood that COVID-19 patients will develop AKI; however, a number of other studies have provided contradictory evidence, casting doubt on this theory (Shi et al., 2021; Zeng et al., 2021; Yarijani and Najaf i, 2021; Liao et al., 2022; Olczak-Pruc et al., 2022; Haroun etal., 2023).

S. Pathophysiology of COVID-19-Induced AKI

The pathophysiology of COVID-19-induced acute kidney injury (AKI) is thought to include localized and systemic inflammatory and immune responses, deterioration of the endothelium, and activation of coagulation mechanisms and the renin-angiotensin system (Tsatsakis et al., 2020; Elmaaty et al., 2021). Direct viral infection with specific kidney targeting of the virus has also been suggested, but remains speculative (Elmaaty et al., 2021). Coronavirus disease 2019 (COVID- 19)-associated acute respiratory distress syndrome involves localized inflammation with the infiltration of immune cells, such as macrophages, effector T cells, and polymorphonuclear neutrophils. Cytokines are released locally within the lung in reaction to damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), exacerbating inflammatory cell recruitment and tissue damage. Immune cells secrete interferon (IFN) to facilitate viral eradication. Neutrophil extracellular snares (NETs), emitted by stimulated neutrophils, could further participate in the localized inflammatory response, elimination of pathogens, and thrombosis. Acute respiratory distress syndrome possibly aids in the emergence of acute kidney injury through systemic methods (e.g., venous congestion and reduced cardiac yield due to right-sided heart failure, high intrathoracic pressure, and hypoxia). Enhanced renal interstitial pressure on account of tissue edema is also likely to contribute to tubular damage. The liberation of danger-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) into the circulatory system abets in causing situated inflammation within the kidneys, ignites an immune system reaction, and advances immune- mediated thrombosis. Specific patients have exhibited direct infection of kidney cells, which might additionally contribute to inflammation in the area and kidney impairment. Conversely, acute kidney injury in diverse scenarios has been evidenced to exacerbate lung injury. This happens by bolstering inflammation in the lung region, heightening the permeability of lung capillaries, and culminating in fluid accumulation. (Al-Karmalawy et al., 2021).

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T. Bilirubin

Bilirubin, an orange-yellow pigment found in bile, is produced through the breakdown of various heme-containing proteins, particularly during the catabolism of hemoglobin. Initially, heme is converted into bi liverd in, which then transforms into unconjugated or indirect bilirubin (UCB). UCB, being water- insoluble, binds to albumin and enters circulation. Within the liver, glucuronic acid is added to unconjugated bilirubin through conjugation, rendering it water-soluble (direct bilirubin). Subsequently, it is either excreted into bile or re-enters the bloodstream, where it undergoes filtration by the kidneys and is excreted through urine (Hanafy and Abd-Elsalam 2020). Elevated plasma bilirubin levels are commonly observed in both primary and hospital care settings. Any liver injury leads to a decrease in hepatocyte count, potentially causing hyperbilirubinemia (Fevery, 2008). This condition may result from abnormalities at various stages of bilirubin metabolism, including excessive production, impaired liver uptake, conjugation defects, or defects in b iliary excretion (Fevery, 2008). While bilirubin is a well-established marker routinely included in biochemical tests for patients with liver dysfunction or other conditions, it lacks sensitivity and specificity as a marker of liver function. Therefore, careful interpretation of test results is essential for accurate diagnosis, considering patient history, the magnitude of the alteration, and concurrent biochemical changes. Elevated bilirubin concentrations can stem from various causes, making it a nonspecific marker of liver dysfunction. Additionally, it is not a sensitive indicator of liver injury; a healthy liver can conjugate daily UCB production twice without increasing total bilirubin concentrations. Moreover, the rate of bilirubin excretion exceeds its production rate by tenfold (Raymond, 1971). Nonetheless, hyperbilirubinemia remains a longstanding marker of liver and bile duct abnormalities, with prognostic significance in certain liver diseases (Stikova et al., 2018).

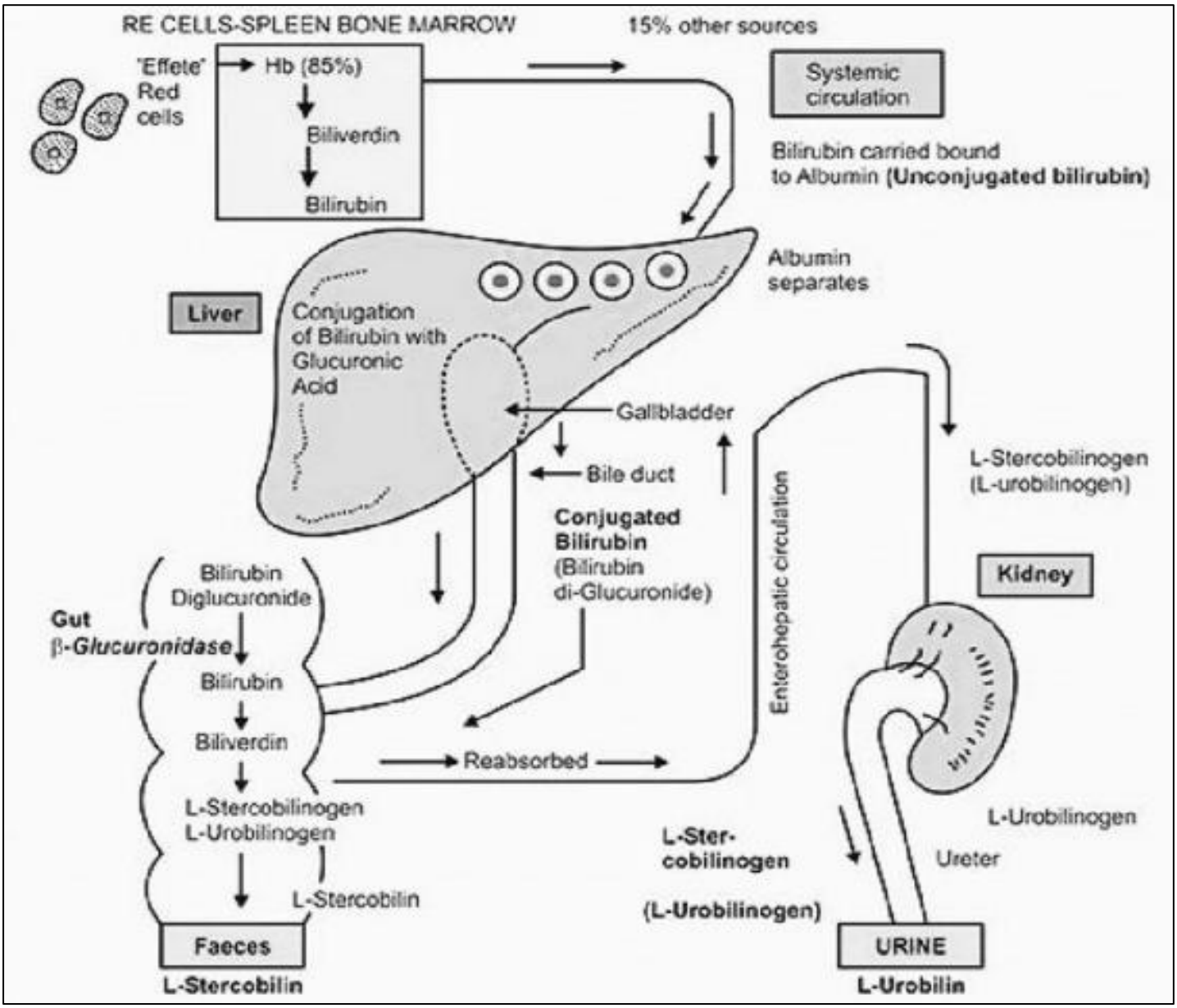

Fig 1: Bilirubin Metabolism in the Liver (Hamidreza, 2022)

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➢ *Laboratory Investigation of Bilirubin*

The reaction of bilirubin with the diazo reagent renders two color azod ipyrroles (azopigments) that can be measured by spectrophotometry, at 530 nm to neutral or acid pH, and 598 nm to alkaline pH (i.e., by the addition of alkaline tartrate). This reaction is accelerated by alcohol and a variety of other components (i.e., sodium benzoate) causing UCB to dissociate from albumin (Fevery, 2008). In the presence of an 'accelerator', conjugated and unconjugated bilirubin are jointly measured (total bilirubin), whereas in the absence of an accelerator, only CB reacts ('direct bilirubin').

The difference between Total and CB yields UCB concentration (' indirect bilirubin'). For the method to be accurate, minimum amounts of UCB must react in the direct procedure. The diazo method described by Jendrassik & Grof in 1938 (Lammert et al., 2008) and later modified by Doumas et al. (Her etal., 2011) yields total serum bilirubin results that are reproducible and reliable. In this method, the accelerator is a caffeine and sodium benzoate solution. This method has acceptable inter-laboratory transferability and is currently the gold-standard method (Fevery, 2008). Its trueness to measure total and direct bilirubin has been assessed by comparing it with UCB and bilirubin diglucuronide quantified by nuclear magnetic resonance.

➢ *Bilirubin Levels in Liver Changes*

In the hyperacute stage of acute liver failure, bilirubin concentration is relatively low as compared to the substantial elevation of plasma aminotransferase concentrations in plasma. However, in the subacute stage, the situation reverses ((Fervery, 2008). In this case, elevated levels of bilirubin in plasma are an indicator of poor prognosis and mortality ((Fervery, 2008). Hyperbilirubinemia does not have a prognostic value in patients with acute hepatitis induced by paracetamol, but it does in acute and subacute hepatitis induced by other causes (Ambrosino et al., 2017). Bilirubin concentrations >17.6 mg/dL is an indication for hospitalization in patients with acute hepatitis unrelated to the intake of paracetamol. Hepatic cirrhosis can be accompanied by progressive bilirubin elevations. Increased bilirubin concentrations are a relatively late event in chronic liver disease and indicate severe liver dysfunction (Fervery, 2008). In acute chronic liver failure, the elevation of bilirubin favors its dissemination across the blood-brain barrier. This situation may be exacerbated by the decrease in albumin concentrations, which impairs bilirubin transport (Fervery, 2008). The consequence is a neurotoxic effect, with progression to a higher level of encephalopathy due to increased concentrations of ammonium ions. High bilirubin concentrations are independent variables associated with the risk of 1- week mortality (Fervery, 2008). Additionally, bilirubin concentrations ≥3.45 mg/dL in patients with chronic liver disease at hospital admission are a predictor of short-term mortality (Zhang et al., 2020). C holestatic liver diseases are characterized by bile flow suppression. Advanced disease causes increased bilirubinemia, generally conjugated (Fervery, 2008). It should not be forgotten that elevation of serum bilirubin does not necessarily indicate liver function status. Indeed, the earliest and most accurate marker of liver failure is prothromb in time measured using the international normalized ratio (INR), which should always be included in the evaluation of acute or chronic liver disease (Ambrosino et al., 2017).

➢ *Effects of the Repurposed Drugs on the Liver Secretory and Synthetic*

- *Function*

Apart from primarily affecting the respiratory system, SARS-CoV-2 also impacts nearly all other organs and systems, leading to myocardial damage, acute coronary syndromes, acute kidney injury, gastrointestinal symptoms, and liver injury. Liver injury emerges as a significant complication in COVID-19 patients, with a transient elevation of transaminases and/or other liver enzymes occurring in approximately 10.5 – 53.1% of cases (Guan et al., 2020). These abnormalities are typically mild to moderate and self-limiting, primarily observed in symptomatic and severe COVID-19 patients (Zhang et al., 2020). Liver function test (LFT) abnormalities generally resolve upon resolution of the COVID-19 infection or discontinuation of hepatotoxic drugs (Pawlotsky et al., 2020). Studies have reported varying degrees of liver injury in COVID-19 patients, with 2 – 11% having pre-existing chronic liver disease and 14– 53% developing hepatic dysfunction, particularly in severe cases. Hepatic dysfunction is notably higher in severe patients and correlates with adverse outcomes (Zhang et al., 2020). Serum bilirubin levels reflect liver secretion capacity, while serum albumin level and prothromb in time indicate liver synthesis capacity. In a study by Zhang et al., severe COVID-19 patients exhibited higher mean total bilirubin levels compared to mild cases (Zhang et al., 2020). The liver serves as a primary site for metabolizing and eliminating chemical substances, including drugs like nucleoside analogs and protease inhibitors, repurposed for COVID-19 treatment. However, drugs used in COVID-19 treatment may exacerbate liver injury, necessitating further evaluation, especially in patients with underlying liver diseases (Sun et al., 2020). Cai et al. demonstrated that patients receiving LPV/r had higher total bilirubin and GGT levels during hospitalization (Cai et al., 2020). Additionally, Sun et al. found that adverse drug events, particularly liver system disorders, were associated with LPV/r and umifenovir in COVID-19 patients. In a meta-analysis, the pooled incidence of drug-induced liver injury among COVID-19 patients was 25.4%, with LPV/r associated with a 37.2% incidence of drug- induced liver injury (Sun et al., 2020).

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Table 1: Changes in Liver Proteins and Enzymes in COVID-19 trials

Drugs	Toxicity	y Type of Toxicity	REFERENCE
LPV/r	8.8%	ALT elevation (>3 ULN)	Cai et al., 2020.
	4.8%	AST elevation (>3 ULN)	
	10.3%	GGT elevation (>3 ULN)	
	2.6%	Total bilirubin elevation (>3 ULN)	
	18.6%	Liver injury	Cai et al., 2020.
	37.2%	Liver injury	Kukarni etal., 2020.
	63.8%	Any adverse drug effect	Sun et al., 2020.
	57.8%	Elevation is more than the ULN value (ALT, AST, ALP, GGT, and total bilirubin)	Fan etal., 2020.
Umifenovir	18.1%	Any adverse drug effect	Sun et al., 2020.
Remdesivir	15.2%	Liver injury	Kukarni etal., 2020.
	3.4%	AST elevation	Beigel et al., 2020
	2.3%	ALT elevation	
	7%	ALT elevation	Goldman et al. 2020
	5.8%	AST elevation	
	32%	AST-ALT elevation	Spinner et al. 2020
	23%	Increased LFTs	Gurein etal. 2020
	10%	Hyperbilirubinemia	Wang et al.2020
	5%	AST elevation	
	2%	ALT elevation leading to discontinuation of Remdesivir	
Hydroxychloroquine	10-fold	Elevation in transaminases	Falcão etal. 2020
Azith romycin	1 –2%	Elevation i,n serum aminotransferases	Her etal. 2020

COVID-19, coronavirus disease 2019; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate transaminase; GGT, gamma-glutamyl transferase; LFT, liver function test; LPV/r, lopinavir/ritonavir; ULN, the upper limit of normal; CPT, Child-Pugh Turcotte; ADEs, adverse drug events.

U. Drug Combinations Therapy

Drug combinations are a mainstay in many areas of medicine, offering several advantages over single-drug therapy, or monotherapy. Combining drugs that target different aspects of a disease process can lead to a more potent therapeutic effect than either drug alone. For example, in HIV treatment, combining multiple antiretroviral drugs can suppress viral replication more effectively than any single drug (Foucquier et al., 2015). Also, by using lower doses of each drug in combination, it's possible to achieve the desired therapeutic effect while minimizing side effects. This is particularly important for drugs that have a narrow therapeutic window, meaning the difference between a safe and effective dose and a toxic dose is small (Toews et al., 2005). Pathogens like bacteria or cancer cells can develop resistance to single drugs. Combining drugs with different mechanisms of action can make it more difficult for resistance to develop (Van Hasselt et al., 2019).

V. Adverse Drug Reactions

An adverse drug reaction (ADR) can be defined as a significant harmful or unpleasant reaction resulting from an intervention related to the use of a medicinal product; adverse effects usually predict hazards from future administration and warrant prevention, specific treatment, or alteration of the dosage regimen, or withdrawal of the product (Aronson et al., 2005).

- *Types of ADRs*

Drug reactions can be categorized as type A which are "Dose-related reactions" (adverse effects at either normal dose or overdose), e.g. serotonin syndrome or anticholinergic effects of tricyclics . Type B reactions are "Non-dose-related reactions" (i.e. any exposure is enough to trigger such a reaction), e.g. allergic or anaphylaxis reactions. Type C is "Dose and time-related reactions" e.g. due to dose accumulation, or prolonged use (e.g. adrenal suppression with corticosteroids). Type D is a "time-related reaction", i.e. due to prolonged use of a drug that doesn't tend to accumulate (e.g. tardive dyskinesia from antipsychotics), while type E is "Withdrawal reactions", i.e. the undesired effects of ceasing the drug (for example, opiate withdrawal). Type F reactions are a result of "Unexpected failure of therapy", where a drug undesirably increases or decreases in efficacy- for example, the decreased clearance of a drug by dialysis, or the decreased effect of antibiotics due to resistance (Edward et al., 2000).

- *Idiosyncratic Reactions*

An idiosyncratic reaction (IDR) refers to an adverse reaction that occurs rarely in patients treated with a drug and does not relate to the drug's intended therapeutic effect. While not the most prevalent type of adverse drug reaction (ADR), IDRs are unpredictable and can be life-threatening. The likelihood of a drug triggering an idiosyncratic reaction depends on its chemical properties, while individual susceptibility is influenced by patient-specific factors, notably the expression of immunologic receptors

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presenting drug-derived antigens on cell surfaces. IDRs pose a significant challenge in drug development because, unless their incidence is high, they often evade detection during clinical trials, leading to instances where serious IDRs prompt the withdrawal of a drug from the market (Uetrecht, 2007). Another characteristic of IDRs is that their risk does not necessarily increase with dosage (Uetrecht, 2007), prompting some to label them as dose-independent. However, no biological effect is truly independent of dosage. Common types of IDRs include skin rash, urticaria, liver injury, and hematologic adverse reactions. Among these, idiosyncratic liver injury (IDILI) stands out as the type most frequently associated with drug withdrawal or black box warnings (Watkins, 2005). This is likely because the liver is a primary site for drug metabolism, often leading to the formation of chemically reactive metabolites. The two predominant forms of IDILI are hepatocellular and c holestatic, though drugs can also induce other types, such as methotrexate- induced liver fibrosis, albeit less commonly. Drugs implicated in IDILI typically generate reactive metabolites in the liver, thought to underlie the adverse reaction (Doyle et al., 2011).

W. Anaphylaxis

Anaphylaxis is a common medical emergency and a life-threatening acute hypersensitivity reaction. It can be defined as a rapidly evolving, generalized, multi-system allergic reaction. Without treatment, anaphylaxis is often fatal due to its rapid progression to respiratory collapse. Historically, anaphylactic reactions were categorized as IgE-mediated responses, while anaphylactoid reactions were categorized as IgE-independent events. Recently, these terms have been consolidated into a single diagnosis of anaphylaxis. Regardless of causation, the resultant clinical is identical (Okubo et al., 2019). Common triggering sources may include exposure to certain medications, foods, or insect stings. Immunotherapy injections directed at improving overall allergic response can induce a hyper-acute reaction (Mota etal., 2018). The mainstay of treatment of acute IgE-mediated or nonimmune anaphylaxis is epinephrine. Epinephrine causes an increase in peripheral vascular resistance plus inotropic and chronotropic cardiac effects, leading to an increase in blood pressure. It causes bronchodilation and decreased mucosal edema through the vasodilation of the skeletal and smooth muscles in the airways and the stabilization of mast cells and basophils (Muraro etal., 2014).

X. Pharmacovigilance

Pharmacovigilance, as defined by the World Health Organization (WHO), encompasses the science and procedures involved in detecting, assessing, understanding, and preventing adverse effects or any other drug-related issues (WHO, 2004). Its pivotal role lies in providing healthcare providers, in collaboration with patients, with comprehensive information to aid in drug selection for treatment decisions (Harmak et al., 2008). Globally, adverse drug reactions (ADRs) stand among the top 10 leading causes of mortality. To mitigate harm to patients, enhance public health, and reduce adverse outcomes, it is essential to establish methods for evaluating and monitoring the safety of medicines used in clinical practice (WHO, 2004). Pharmacovigilance programs also shed light on the potential implications of evolving trends in the field. However, they encounter significant challenges such as globalization, the prevalence of web-based sales and information, broader safety concerns, and the balance between public health and the economic interests of the pharmaceutical industry. Additionally, monitoring established products, addressing the needs of developing and emerging countries, and understanding attitudes and perceptions regarding the balance between drug benefits and risks pose considerable challenges (Biswas et al., 2009). Pharmacovigilance programs serve as essential tools for identifying gaps in our comprehension of medicine-induced diseases and should thus be a priority for every country with public health disease control initiatives (WHO, 2004).

Y. Glucose Metabolism

Glucose is a vital energy source for various cellular processes and plays a crucial role in maintaining homeostasis within the body. Glucose metabolism encompasses a complex network of pathways and processes that govern the synthesis, utilization, and regulation of this essential monosaccharide.

➢ *Pathways of Glucose Metabolism*

- *Glycolysis:*

This cytoplasmic pathway is the primary route for glucose catabolism, converting one molecule of glucose into two molecules of pyruvate, with the concomitant generation of adenosine triphosphate (ATP) and reduced nicotinamide adenine dinucleotide (NADH).

- *Gluconeogenesis:*

This biosynthetic pathway occurs primarily in the liver and, to a lesser extent, in the kidneys, allowing for the synthesis of glucose from non- carbohydrate precursors, such as pyruvate, lactate, glycerol, and certain amino acids.

- Glycogenesis:

This anabolic pathway facilitates the storage of excess glucose as glycogen, primarily in the liver and skeletal muscles. Glycogen acts as a readily available energy reserve.

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- *Glycogenolysis:*

This catabolic process breaks down glycogen into glucose-1- phosphate, which can be converted to glucose-6-phosphate and enter the glycolytic pathway or be released into the bloodstream as free glucose.

- *Pentose Phosphate Pathway (PPP):*

This pathway serves two primary functions: the generation of NADPH, a crucial cofactor in anabolic processes and antioxidant defense mechanisms, and the production of ribose-5-phosphate, a precursor for nucleotide synthesis.

- *Hexosamine Biosynthetic Pathway (HBP):*

This minor pathway utilizes fructose-6-phosphate, a glycolytic intermediate, as a substrate for the synthesis of uridine diphosphate N-acetylglucosamine (UDP-GlcNAc), a precursor for g lycosylation reactions and proteoglycan synthesis.

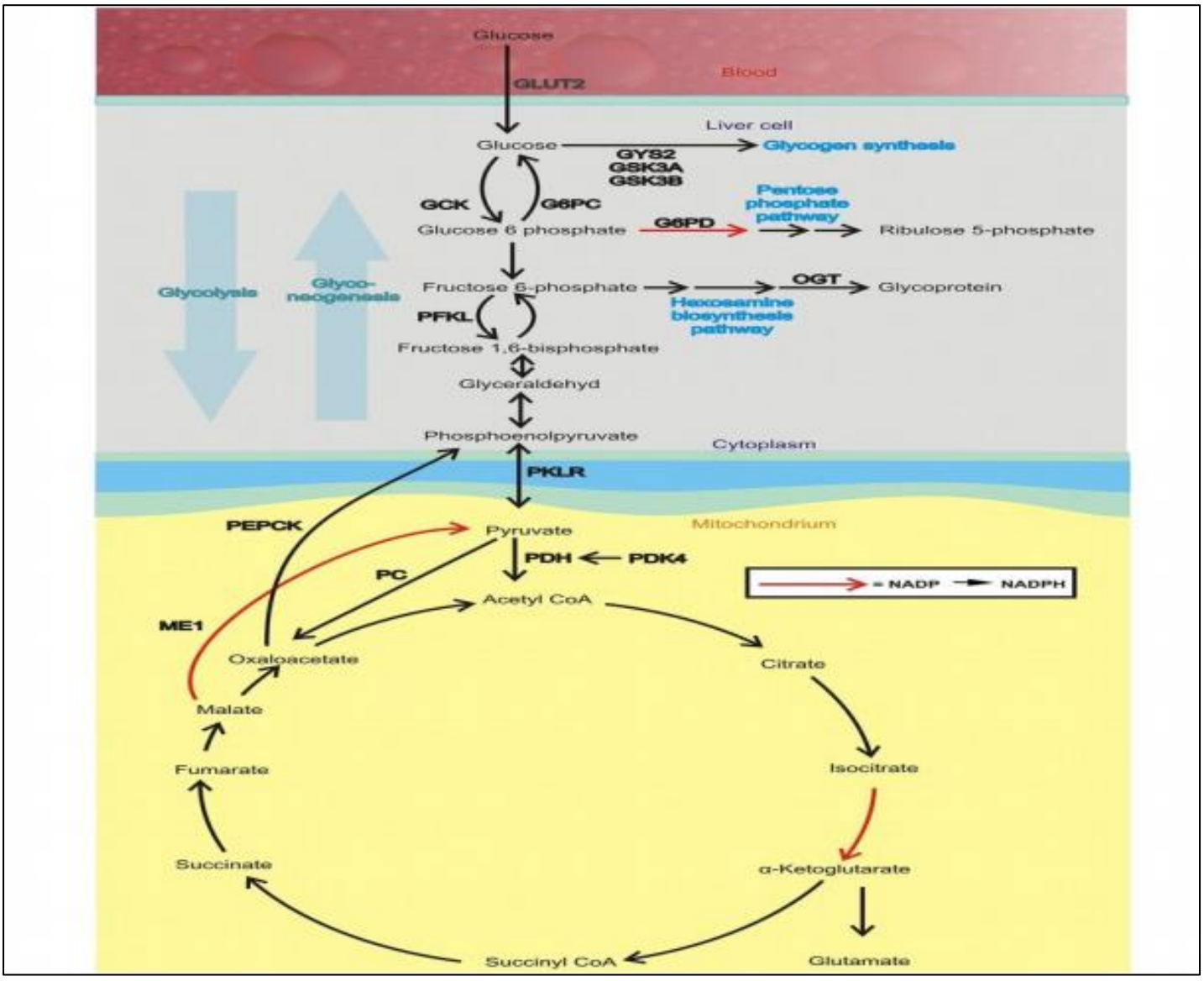

Fig 2: Illustrating Glucose Metabolism Pathways (Lindquist et al., 2019)

➢ *Glucose Synthesis:*

Glucose can be synthesized through various pathways, depending on the available precursors:

- *Gluconeogenesis:*

This process primarily occurs in the liver and involves the conversion of non-carbohydrate precursors, such as pyruvate, lactate, glycerol, and certain amino acids, into glucose.

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- *Glycogenolysi:*

The breakdown of glycogen stores in the liver and skeletal muscles can release glucose-1-phosphate, which can be converted to glucose-6- phosphate and eventually released into the bloodstream as free glucose.

➢ *Glucose as a Marker for Diabetes Mellitus:*

Glucose levels in the blood are closely monitored in the clinical setting as a diagnostic and management tool for diabetes mellitus, a metabolic disorder characterized by persistent hyperglycemia (elevated blood glucose levels).

- *Fasting Blood Glucose (FBG):*

Elevated FBG levels (≥126 mg/dL or ≥7.0 mmol/L) after an overnight fast are indicative of diabetes mellitus.

- *Oral Glucose Tolerance Test (OGTT):*

This test measures blood glucose levels after consuming a standardized glucose solution. Impaired glucose tolerance or diabetes mellitus is diagnosed based on the 2-hour post-load glucose values.

- *Glycated Hemoglobin (HbA1c):*

This test provides an estimate of average blood glucose levels over the past 2-3 months by measuring the amount of hemoglobin that has been glycated (bound to glucose). HbA1c levels ≥6.5% are indicative of diabetes mellitus.

➢ *Hyperglycemic and Hypoglycemic Condition:*

Glucose homeostasis is tightly regulated by various hormones, including insulin and glucagon, as well as by the actions of various tissues and organs, such as the liver, pancreas, and skeletal muscles.

- *Hyperglycemia:* Elevated blood glucose levels can result from various factors, including:

✓ Impaired insulin secretion or action (as in diabetes mellitus)
✓ Excessive gluconeogenesis or glycogenolysis
✓ Certain medications (for example, corticosteroids, protease inhibitors)
✓ Stress or illness

- *Hypoglycemia:* Decreased blood glucose levels can be caused by:

✓ Excessive insulin administration or secretion
✓ Impaired g luconeogenesis or glycogenolysis
✓ Malnutrition or starvation
✓ Certain medications (for example, sulfonylureas, insulin sensitizers)
✓ Hormonal imbalances (for example, excess insulin-like growth factor-1, adrenal insufficiency)

➢ *Regulation of Glucose Metabolism:*

Glucose metabolism is tightly regulated by several hormones and signaling pathways, ensuring a balance between glucose uptake, utilization, and storage:

- *Insulin:*

Secreted by the pancreatic β-cells, insulin promotes glucose uptake by facilitating the translocation of glucose transporters (GLUT4) to the cell membrane in skeletal muscles and adipose tissue. It also stimulates glycogen synthesis and inhibits gluconeogenesis and glycogenolysis.

- *Glucagon:*

Produced by the pancreatic α-cells, glucagon acts as a counterregulatory hormone to insulin, promoting glucose release by stimulating glycogenolysis and g luconeogenesis in the liver.

- *Epinephrine and Glucocorticoids:*

These hormones enhance gluconeogenesis, glycogenolysis, and lipolysis, thereby increasing blood glucose levels during stress or fasting conditions.

- *Incretin Hormones (GLP 1 and GIP):*

These hormones, secreted by the gastrointestinal tract, enhance insulin secretion and inhibit glucagon release in response to nutrient intake, contributing to glucose homeostasis.

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Glucose metabolism is a complex and tightly regulated process, involving intricate interactions between various pathways, tissues, and hormones. Maintaining glucose homeostasis is crucial for overall health and metabolic function, and dysregulation of glucose metabolism can lead to various metabolic disorders, including diabetes mellitus. Continued research and understanding of glucose metabolism are essential for developing effective therapeutic strategies and improving patient car.

➢ *Potential Mechanisms by which COVID-19 Drugs-19 May Affect Glucose*

- *Levels*

COVID-19 drugs may affect glucose homeostasis through the following direct or indirect mechanisms:

✓ *Direct Effects on Glucose Metabolism:*

- Some COVID-19 drugs may interfere with the insulin signaling pathway, leading to insulin resistance or impaired glucose uptake by cells (Bastard et. al. 2006).
- Certain drugs may affect the function or expression of key enzymes involved in glucose metabolism, such as glucokinase or glucose-6-phosphatase (Postic et. al. 2001).
- Drugs may alter the secretion or sensitivity to hormones like insulin or glucagon, which play crucial roles in glucose regulation (Goldfine et. al. 1972).

✓ *Indirect Effects:*

- COVID-19 drugs may induce inflammation or stress responses, which can lead to insulin resistance and dysregulated glucose homeostasis (Lontchi-yachen et. al. 2021).
- Some drugs may affect the gut microbiome, which has been linked to glucose metabolism and insulin sensitivity (Sohail et. al. 2017).
- Drug-induced liver or kidney dysfunction could impair glucose homeostasis by altering gluconeogenesis or glucose excretion (S ku be et. al. 2021).

➢ *Previous Studies on Covid-19 Drugs and Glucose Dysregulation*

While research in this area is still limited, several studies have reported glucose dysregulation as a potential side effect of certain COVID-19 drugs. For example, a clinical trial investigating the use of corticosteroids in COVID-19 patients found an increased risk of hyperglycemia and new-onset diabetes (Rubino et. al. 2020). Another study reported cases of severe hypoglycemia in COVID-19 patients treated with remdesivir, an antiviral drug (Hur et. al. 2021)

Preclinical studies in animal models have also provided insights into the potential mechanisms by which COVID-19 drugs may affect glucose homeostasis. For instance, a study in mice found that treatment with certain antivirals disrupted insulin signaling and led to insulin resistance (Yang et. al. 2021).

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CHAPTER THREE
MATERIALS AND METHODS

A. Study Area

The study was conducted at the Departments of Chemical Pathology at the University of Benin Teaching Hospital in Benin City and pharmacology and toxicology at the University of Benin in Nigeria.

B. Materials

The following items are needed: A Centrifuge (Rolotix 32A Germany), a Spectrophotometer, an Orogastric tube, Cotton wool, Napkins, permanent Markers, test tubes, surgical Gloves, surgical dissection kits, plain sample vials and Cotton wool.

C. Ethical Consideration

Ethical approval was obtained from the Edo State Ministry of Health. (protocol number.: HA/737/24/C/0708320).

D. Animal Care

Wistar Rats weighing between 100 and 200g were obtained from the facility that houses animals at the University of Benin's Department of Pharmacology and Toxicology, which is located in Benin City, Nigeria. The rodents were housed in cages made of plastic for two weeks to acclimate to their new surroundings, which included plastic cage bedding with wood shavings and conditions that were maintained at a consistent temperature (25ºc +_ 3ºc) and a natural light- dark cycle that alternated every 12 hours. The rodents were given commercial rodent feed that was pelletized and finished, and they had constant access to water. The wood shavings that were used as bedding in the cages were cleaned and replaced daily. All of the experiments that were carried out were done under the rules that were established by the National Institute of Health for the care and use of animals that are utilized in Laboratory settings.

E. Drugs and Chemicals

Reputable manufacturers supplied registered brand-name medications (Chloroquine, Hydroxychloroquine, Ivermectin, Lopinavir/Ritonavir, Azith romycin, Zinc/Selenium), which were acquired from a licensed pharmacy. Specific doses of each medication were calculated based on the rats' body weight and dissolved in distilled water for oral administration using an oral gastric tube. The research laboratory's departmental reagent store provided concentrated hydrochloric acid (HCL), sodium citrate, sodium bicarbonate, and other essential chemicals.

F. Experimental Design

60 Wistar rats were randomly selected into 10 groups, a control group and 9 experimental groups (1, 2, 3, 4,5, 6, 7, 8, 9) with each containing 6 rats. The experimental animals were treated orally with freshly prepared drug (dissolved in 0.5ml of water), while the control group was administered water for twenty-eight days. The following were the treatments orally to each group:

- *(CON) - Six rats were administered distilled water as a control*

- Group 1: (CQ) - Six rats were administered a calculated therapeutic dose of Chloroquine dissolved in water
- Group 2: (HCQ) - Six rats were administered a calculated therapeutic dose of Hydroxychloroquine dissolved in water
- Group 3: (IV) - Six rats were administered a calculated therapeutic dose of Ivermectin dissolved in water
- Group 4: (LR) - Six rats were administered a calculated therapeutic dose of Lopinavir/Ritonavir dissolved in water.
- Group 5: (AZ) - Six rats were administered a calculated therapeutic dose of Azith romycin dissolved in water
- Group 6: (ZNSE) Six rats were administered a calculated therapeutic dose of Zinc/Selenium dissolved in water.
- Group 7: Six rats were administered a calculated combined therapeutic dose of Chloroquine, Ivermectin, Lopinavir/Ritonavir, Azith romycin, Zinc/Selenium dissolved in water
- Group 8: Six rats were administered a calculated combined therapeutic dose of Hydroxyl Chloroquine, Ivermectin, Lopinavir/Ritonavir, Azith romycin, Zinc/Selenium dissolved in water
- Group 9: Six rats were administered a calculated combined therapeutic dose of Ivermectin, Lopinavir/Ritonavir, Azith romycin, and Zinc/Selenium dissolved in water.

G. Determination of Change in Body Weight

The weight of the animals was taken before drug administration, after which at every seven days intervals the animals were re-weighed and the doses adjusted in response to a change in weight and at the completion of the twenty-eight days drug administration period using the Scout pro digital balance (OHAUS corporation, USA), the final weight of the rats is determined.

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H. Number of Rat Per Group

- Total number of animals receiving Chloroquine= 6 rats
- Total number of animals receiving Hydroxychloroquine= 6 rats
- Total number of animals receiving Ivermectin = 6 rats
- Total number of animals receiving Lopinavir/Ritonavir = 6 rats
- Total number of animals receiving Azith romycin= 6 rats
- Total number of animals receiving Zinc/Selenium= 6 rats
- Total number of animals receiving Chloroquine, Ivermectin, Lopinavir/Ritonavir, Azith romycin, Zinc/Selenium = 6 rats
- Total number of animals receiving Hydroxychloroquine, Ivermectin, Lopinavir/Ritonavir, Azith romycin, Zinc/Selenium = 6 rats
- Total number of animals receiving Ivermectin, Lopinavir/Ritonavir, Azith romycin, Zinc/Selenium = 6 rats.

I. Dosage Calculations

Therapeutic dose calculation

Volume of 0.5mL of drug Administered

If 200g Rat = 0.5mL

1g = 1/200 X 0.5 = 0.0025

Therefore, therapeutic dose = Weight X 0.0025

Therapeutic dose calculation for Chloroquine in cage 1

0ne tail marked rat; 166g (male) X 0.0025 = 0.42mL

Two tail marked Rat; 173g (F) X 0.0025 = 0.43mL

Three tail marked Rat; 196g(M) X 0.0025 = 0.49 mL

Four tail marked Rat; 136(M) X 0.0025 = 0•34mL

Five tail marked Rat; 186(F) X 0.0025 == 0.47mL

Six tail marked Rat; 148 (F) X 0.0025= 0.37mL

Therapeutic dose calculation for Hydroxychloroquine in cage 2

One tail marked rat; 196g (F) X 0.0025 = 0.48mL

Two tail marked Rat; 161g (M) X 0.0025 = 0.40 mL

Three tail marked Rat; 165g (F) X 0.0025 = 0.41mL

Four tail marked Rat; 140g (M) X 0.0025 = 0.35mL

Five tail marked Rat; 128g (M) X 0.0025 = 0.32mL

Six tail marked Rat; 160g (M) X 0.0025 = 0.4mL

Therapeutic dose calculation for Ivermectin in cage 3

0ne tail marked rat; 171g(M) X 0.0025 = 0.43mL

Two tail marked Rat; 192g(M) X 0.0025 = 0.48Ml

Three tail marked Rat;164g(F) X 0.0025 = 0.39mL

Four tail marked Rat; 155g(F) X 0.0025 = 0.39mL

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Five tail marked Rat; 164g(F) x 0.0025 = 0.41mL

Six tail marked Rat; 132g(M) x 0.0025 = 0.33mL

Therapeutic dose calculation for Lopinavir/Ritonavir in Cage 4

0ne tail marked rat; 122g(F) X 0.0025= 0.31mL

Two tail marked Rat; 110g(F) X 0.0025= 0.27mL

Three tail marked Rat;152g(F) X 0.0025= 0.38mL

Four tail marked Rat;163g(F) X 0.0025= 0.41mL

Five tail marked Rat; 126g(F) X 0.0025= 0.32mL

Six tail marked Rat; 133g(F) X 0.0025= 0.33mL

Therapeutic dose calculation for Azith romycin in Cage 5

0ne tail marked rat; 188g(M) X 0.0025 = 0.295mL

Two tail marked Rat; 153g(F) X 0.0025 = 0.383mL

Three tail marked Rat;184g(M) X 0.0025 = 0.46mL

Four tail marked Rat;125g(F) X 0.0025 = 0.313mL

Five tail marked Rat; 144g(M) X 0.0025 = 0.36mL

(Unmarked); 127g(M) X 0.0025 = 0.3175mlmL

Therapeutic dose calculation for Zinc/Selenium in cage 6

0ne tail marked rat; 170g(F) X 0.0025 = 0.425mL

Two tail marked Rat; 143g(M) X 0.0025 = 0.358mL

Three tail marked Rat;144g(F) X 0.0025 = 0.36mL

Four tail marked Rat;134g(M) X 0.0025 = 0.335mL

Five tail marked Rat; 145g(M) X 0.0025= 0.363mL

Six tail marked Rat; 125g(M) X 0.0025= 0.3125mL

Black faeces was observed in this cage on day 13

Therapeutic dose calculation for Chloroquine, Ivermectin, Lopinavir/Ritonavir, Azith romycin & Zinc/Selenium in cage 7

0ne tail marked rat; 108g(M) X 0.0025 = 0.27mL

Two tail marked Rat; 131g (M) X 0.0025 = 0.33mL

Three tail marked Rat;117g(F) X 0.0025 = 0.30mL

Four tail marked Rat;140g(F) X 0.0025 = 0.373mL

Five tail marked Rat; 170g(F) X 0.0025 = 0.425mL

Six tail marked Rat; 130g(F) X 0.0025 = 0.325mlmL

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Therapeutic dose calculation for Hydroxyl Chloroquine, Ivermectin, Lopinavir/Ritonavir, Azith romycin & Zinc/Selenium in cage 8

0ne tail marked rat;153g (M) X 0.0025 = 0.383mL

Two tail marked Rat; 164g(F) X 0.0025 = 0.41mL

Three tail marked Rat;178g(F) X 0.0025 = 0.45ml Four tail marked Rat;191g(F) X 0.0025 = 0.48mL

Five tail marked Rat;159g(F) X 0.0025 = 0.40mL

Three tail marked Rat;166g(F) X 0.0025 = 0.42mL

Therapeutic dose calculation for Ivermectin, Lopinavir/Ritonavir, Azith romycin & Zinc/Selenium in cage 9

0ne tail marked rat;137g(M) X 0.0025 = 0.34mL

Two tail marked Rat;146g(F) X 0.0025 = 0.37mL

Three tail marked Rat;138g(M) X 0.0025 = 0.35mL

Four tail marked Rat;160g(F) X 0.0025 = 0.4mL

Five tail marked Rat;203g(F) X 0.0025 = 0.5mL

Six tail marked Rat;138g(M) X 0.0025 = 0.35mL

J. Preparation and Administration of Drugs

Calculated standard doses were computed in kilogram/body weight. The tablets were grounded into powder and triturated using water. The solution was transferred into a previously calibrated container and made up to volume. The final preparation was administered using orogastric tube into the Wistar rats based on the corresponding grouping. They were administered the freshly prepared drugs for twenty-eight days. The animal was anesthetized using chloroform and sacrificed. Blood samples were collected and assayed for the traditional toxicity profile as described (Ramirez et al., 2021).

K. Evaluation of Toxicity Markers in Rats

After 4 weeks of treatment, the rats were excised after humane sacrifice of the animals under chloroform anesthesia. Using a pair of surgical scissors, the deeply anesthetized rat was dissected carefully. Then using a sterile 5 ml syringe with a 23G needle, about 2 ml of blood was withdrawn from the retro-orbital sinus vein and heart of each rat and transferred to pre-labeled lithium heparin (blue) tubes. Blood samples were first centrifuged at 40 rpm/minute for 3 minutes. The plasma (clear portion) was carefully collected into pre-labeled plain (red color) tubes using different sterile syringes for each, and the serum was discarded.

L. Biochemical Analysis

➢ *Urea*

Plasma Urea Estimation by Urease-Berthelot Reaction (Ochei and Kolhatkar, 2019)

- Principle: Principle: The enzyme urease hydrolyses urea at 37C. The ammonia produced reacts with alkaline hypochlorite and phenol in the presence of a catalyst to form indophenol which is measured at 550nm spectrophotometrically.

➢ *Test Procedure*

- 100µl of urease reagent was pipetted into the sample test, standard, and blank tubes respectively
- 0µl of the sample was added to the tube marked sample test
- 10µl of the standard was added to the tube marked standard
- Distilled water was added to the tube marked blank
- The tubes were mixed and incubated at 37°C for 10mins
- 2000µl phenol reagent was added to all the tubes
- 2000µl hypochlorite solution was added to all the tubes
- The tubes were mixed and incubated at 37°C for 15mins. Absorbance was read at 550nm against a reagent blank

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➢ *Calculation:*
Urea concentration (mg/dl) = Absorbance of test X Concentration of standard (80mg/dl Absorbance of standard

➢ *Creatinine*
Plasma Creatinine Estimation by the Jaffe-Slot Alkaline Pirate Creatine Method (Ochei and Kolhatkar, 2019)

- Principle: Creatinine in alkaline medium, reacts with picnic acid to form Picrates. The intensity of the yellow colour complex formed is proportional to the concentrations of creatinine in the sample and is read at a wavelength of 500nm.

➢ *Test Procedure:*

- 1000µl of picnic acid was pipetted into test tubes labeled sample, standard and blank respectively.
- 1000µl alkaline solution was added to all the tubes
- 100µl of the sample was added to the sample test
- 100µl of the standard was added to the tube marked standard
- The tubes were mixed and incubated at room temperature for 10mins.
- Absorbance was read at 500nm against the reagent blank.

➢ *Calculation:*
Creatinine Conc. (mg/dl) = Absorbance of test X Standard Conc. Absorbance of standard.

M. Glucose Estimation

➢ *Oxidase-Peroxidase Method (Ochei and Kolhatkar, 2019)*
The oxidase-peroxidase method, also known as the glucose oxidase-peroxidase (GOD-POD) method, is a widely used and reliable technique for estimating glucose levels in biological samples, such as blood, plasma, or serum. This method is based on a series of enzymatic reactions that couple the oxidation of glucose-by-glucose oxidase (GOD) with the subsequent reduction of a ch romogenic substrate by peroxidase (POD) in the presence of hydrogen peroxide (H2O2).

➢ *Principle*
The oxidase-peroxidase method for glucose estimation involves the following Sequential Reactions:

- *Glucose Oxidation*
Glucose + O2 + H2O ----------> G luconic acid + H2O2(Glucose Oxidase)

- *Chromogen Oxidation:*
H2O2 + Ch romogen (reduced) ----------> Ch romogen (oxidized) + H2O(Peroxidase)

In the first step, glucose oxidase (GOD) catalyzes the oxidation of glucose to g luconic acid, producing hydrogen peroxide (H2O2) as a byproduct. In the second step, the hydrogen peroxide reacts with a ch romogenic substrate (e.g., o- dianisidine, 4-amino antipyrine, or 2,2'-azino-b is (3-ethylbenzothiazoline-6- sulfonic acid) (ABTS)) in the presence of peroxidase (POD). This reaction results in the oxidation of the ch romogen, producing a colored compound that can be measured spectrophotometrically.

The intensity of the color produced is directly proportional to the concentration of glucose in the sample, allowing for quantitative analysis based on Beer's law.

N. Assay of Bilirubin (Ochei and Kolhatkar, 2019)

➢ *Principle of Test*
Bilirubin is determined in the presence of caffeine by the reaction with diazotized sul phanilic acid to produce an intensely coloured diazo dye. This complex is measured spectrophotometrically and the intensity is directly proportional to the total bilirubin present.

➢ *Procedure*

- The tubes for the test, blank and standard were set up together.
- 10uL of sample was added to the test tube
- 10uL of distilled water was added to blank
- 3mL of BCG was added to the test, standard and blank.

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- The tubes were incubated for 5 minutes at room temperature and read
- spectrophotometrically at a wavelength of 639nm.

➢ *Calculation*

Serum bilirubin concentration = Absorbance of test/Absorbance of standard × concentration of standard (g/dL).

➢ *Reference Range*

- TB: 0.2-1.2mg/dl
- CB: 0.1-0.6mg/dl

O. Quality Control

- Proper identification of albino rats was done using cage cards to differentiate between albino rats across the various study group
- Albino rats within the same cage were differentiated from each other by coloured stains
- Analytical grades of reagents were used throughout the research process.
- Reagents were checked for expiry date before use.
- The equipment was calibrated properly
- The stability of calibration was checked periodically.
- Standards of various parameters were subjected to various test methods in order to check the reliability of the data.

P. Statistical Analysis

The information was entered into Graph Prism Version 6 in San Diego, California. As Mean variants, they were calculated. One-way ANOVA (Analysis of Variance) was used to apply inferential statistics. P-values equal to or less than 0.05 were used to determine statistical significance.

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CHAPTER FOUR
RESULTS

Table 2 Mean glucose levels in the control group and various treatment groups highlighted significant differences in glucose metabolism among certain treatments. Particularly noteworthy is the HCQ + AZI + L.R + IV + Zn + Se group, which showed a markedly elevated mean glucose level of 103.80 mg/dL (P = 0.001), indicating a statistically significant impact on glucose metabolism. Conversely, the Lopinavir/Rotinavir group exhibited a relatively lower mean glucose level of 83.33 mg/dL, with no significant difference compared to the control (P = 0.924), suggesting minimal influence on glucose levels. Furthermore, the Zinc/selenium group, with a mean glucose level of 78.6 mg/dL, showed no significant difference compared to the control (P = 0.328).

Table 2: Mean Glucose Levels of the Control Group and the Various Treatment Groups

Treatment Groups	**Treatment Glucose (mg/dL)**	**Control Glucose(mg/dL)**	**P-value**
Azith romycin	Mean	±SEM	0.328
	89.00±3.225	83.83±4.549	
Chloroquine	88.83±4.453		0.343
CQ + IV + L.R + AZI + Zn + See	87.33±4.667		0.587
HCQ + AZI + L.R + IV + Zn + Se	103.80±2.131		0.001*
Hydroxychloroquine	92.33±5.333		0.111
IV + L.R + AZI + Zn + Se	92.50±4.093		0.145
Ivermectin	86.80±2.956		0.590
Lopinavir/Ritonavir	83.33±1.430		0.924
Zinc/selenium	78 6 ±3 964		0 328

*=p-Value Significant at ≤0.05; SEM= Standard Error of Mean

➢ *Key*

- SEM=Standard error of the mean
- CQ=Chloroquine
- HCQ=Hydroxychloroquine
- AZI=Azith romycin
- IV=Ivermectin
- L.R=Lopinavir/Ritonavir
- ZN/SE=Zinc/Selenium

Figure 3 presents a simple bar chart showing the mean glucose levels of both the control group and the various treatment groups. The effect of each drug on the glucose level is illustrated by the simple bar chart.

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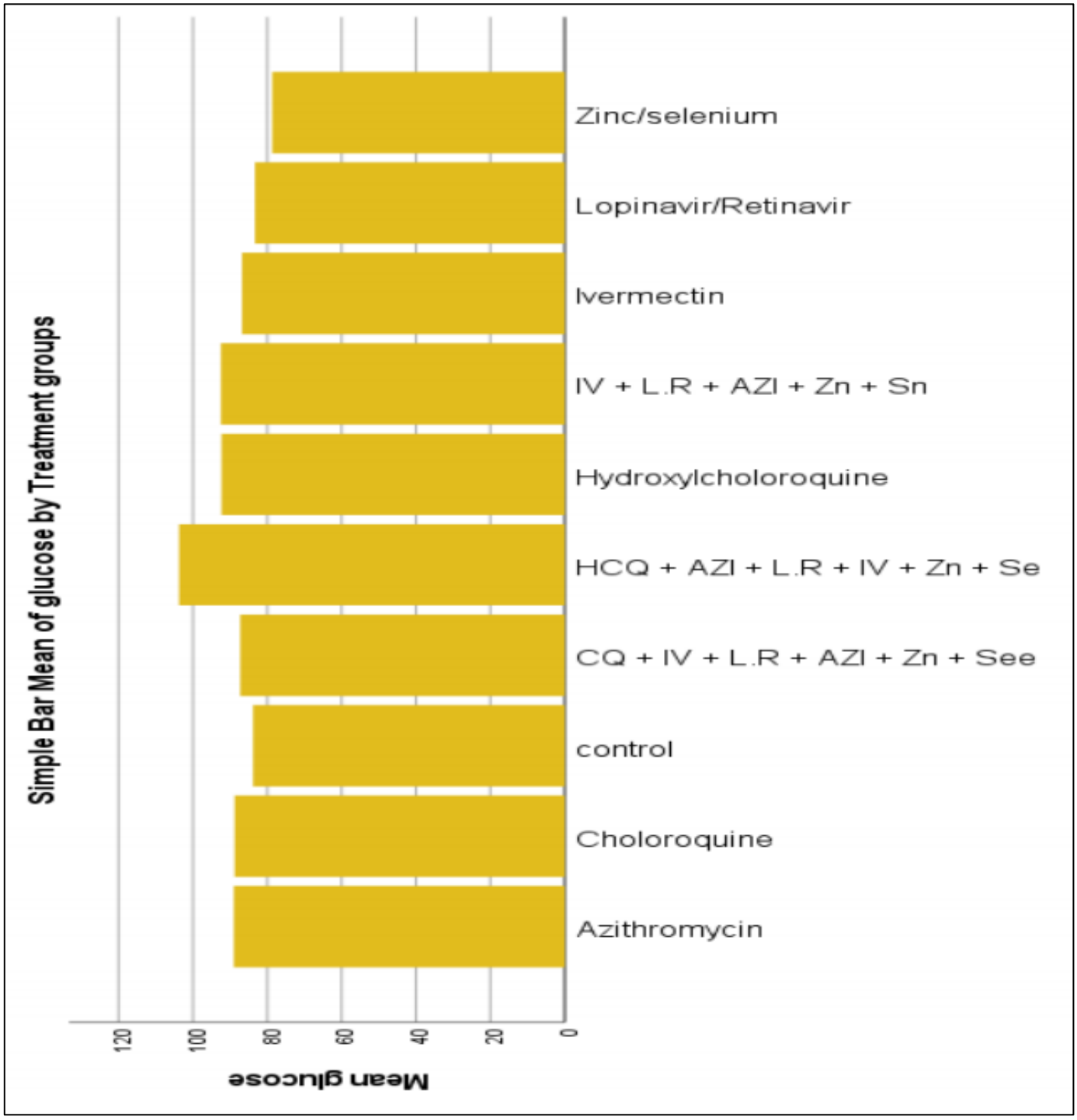

Fig 3: Showing Effect of Each Drug on the Glucose Level

Table 3 presents urea and creatinine levels of both the control group and the various treatment groups. With regard to urea levels, there was no discernible variation was seen between the groups ($F = 1.741$, $p = 0.109$), suggesting that there was no significant variation in the groups' mean urea levels ($p>0.05$).

In the same way, there was no discernible variation in creatinine levels across the treatment groups ($F = 0.525$, $p = 0.848$), indicating that there was no significant difference in the groups' mean creatinine levels ($p>0.05$).

Notably, groups 7 and 8 displayed significant differences in urea and creatinine levels relative to control, albeit not statistically significant differences in zinc and selenium levels.

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Table 3: Levels of Urea and Creatinine in all Study Groups

Drugs	Urea	Creatinine
CON	41.00±4.91	0.62±0.17
CQ	48.17±2.80	0.50±0.15
HQ	56.50±5.88	0.60±0.10
IV	50.20±5.70	0.54±0.11
LR	49.50±1.52	0.65±0.32
AZ	53.17±1.58	0.50±0.11
ZNSE	38.33±6.30	0.47±0.10
7	38.33±3.38	0.33±0.03
8	41.60±5.81	0.34±0.02
9	46.25±6.46	0.45±0.06
F	1.741	0.525
P	0.109	0.848

➢ *Key*

- CON - Control
- CQ - Chloroquine
- HCQ - Hydroxychloroquine
- IV - Ivermectin
- L/R - Lopinavir-ritonavir
- AZ - Azith romycin
- ZNSE - Zinc and Selenium
- Chloroquine, Ivermectin, Lopinavir/Ritonavir, Azith romycin, Zinc/Selenium
- Hydroxychloroquine, Ivermectin, Lopinavir/Ritonavir, Azith romycin, Zinc/Selenium
- Ivermectin, Lopinavir/Ritonavir, Azith romycin, Zinc/Selenium

Figure 4 illustrates a bar chart showing the mean urea levels among all groups , highlighting there was no statistically significant variation in the average urea levels between the different treatments.

Figure 5 presents a bar chart showing the mean creatinine levels among all groups , highlighting there was no statistically significant variation in the average creatinine levels between the different treatments.

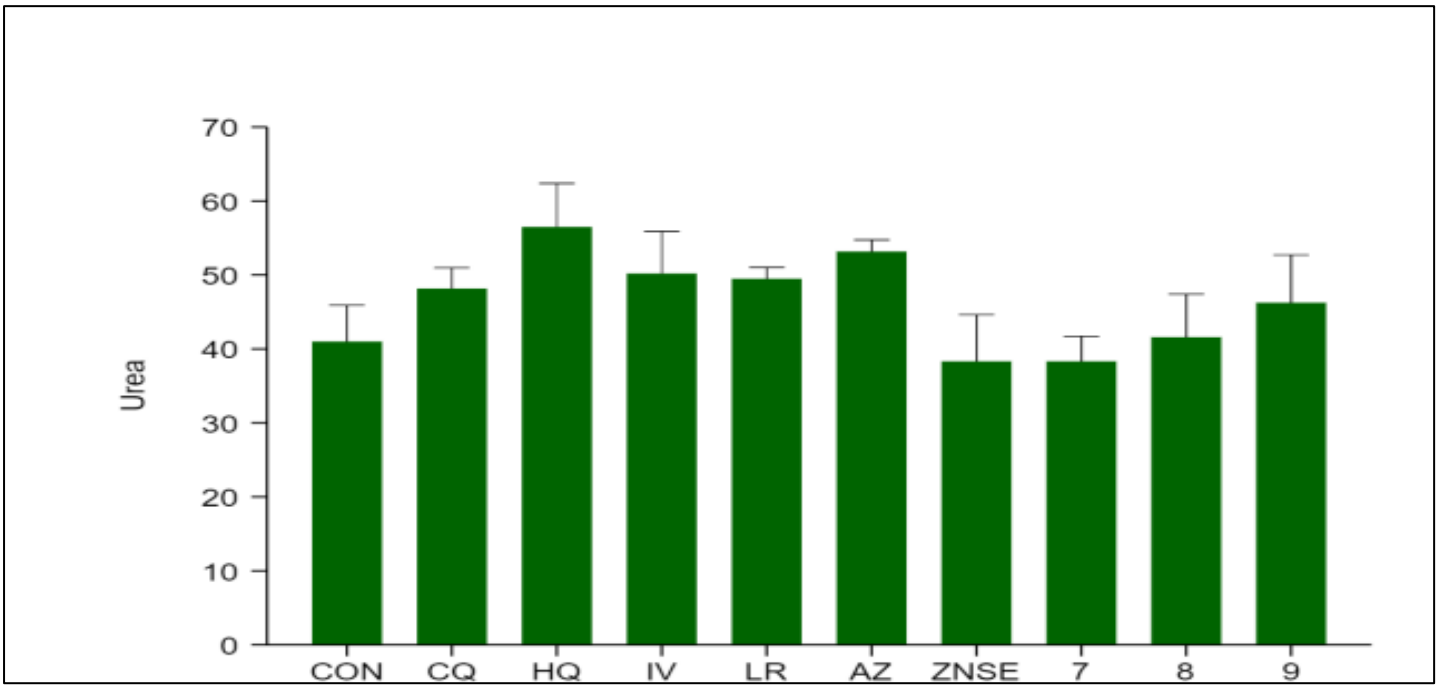

Fig 4: Bar Chart Showing Mean Urea Levels among a Group

There was no statistically significant variation in the average urea levels between the different treatments

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➢ *Key*

- CON - Control
- CQ - Chloroquine
- HCQ - Hydroxychloroquine
- IV - Ivermectin
- L/R - Lopinavir-ritonavir
- AZ - Azith romycin
- ZNSE - Zinc and Selenium
- Chloroquine, Ivermectin, Lopinavir/Ritonavir, Azith romycin, Zinc/Selenium
- Hydroxychloroquine, Ivermectin, Lopinavir/Ritonavir, Azith romycin, Zinc/Selenium
- Ivermectin, Lopinavir/Ritonavir, Azith romycin, Zinc/Selenium

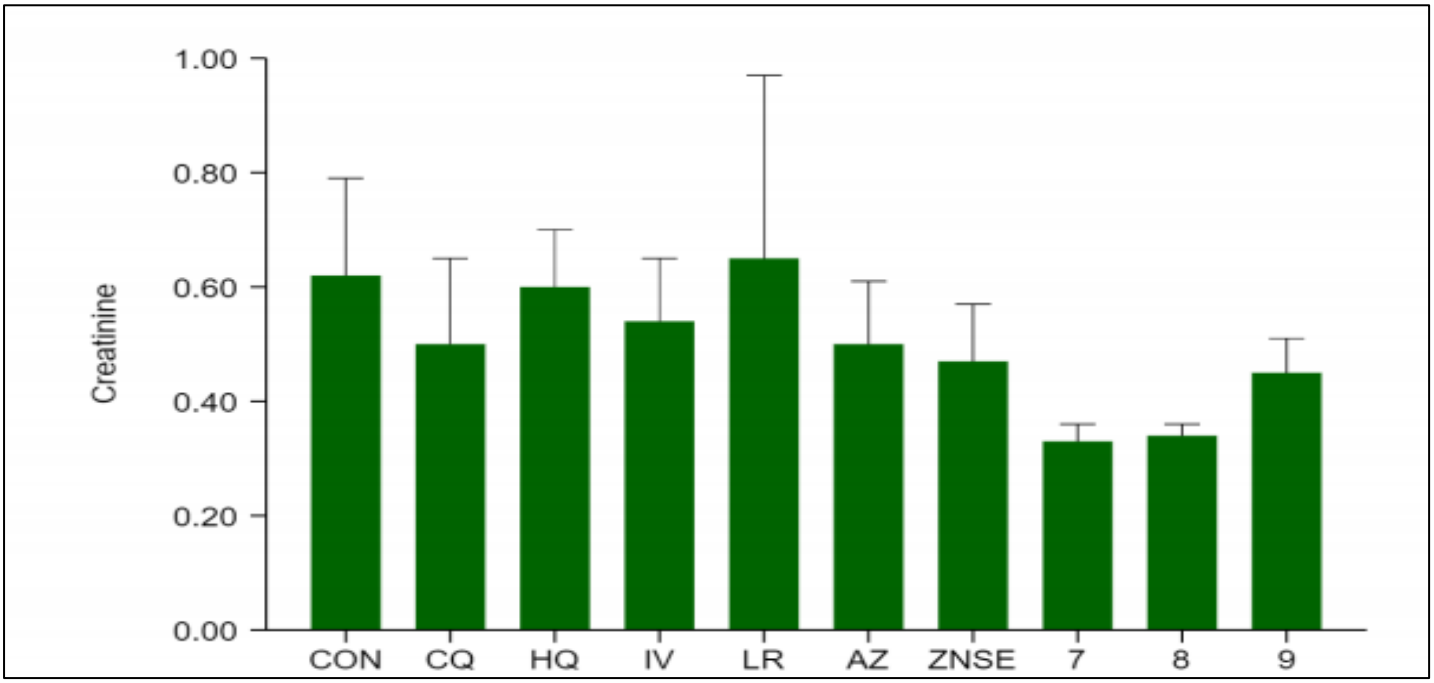

Fig 5: Bar Chart Showing Mean Creatinine Levels among All Groups

➢ *Key*

- CQ - Chloroquine
- HCQ - Hydroxychloroquine
- IV - Ivermectin
- L/R - Lopinavir-ritonavir
- AZ - Azith romycin
- ZNSE - Zinc and Selenium
- Chloroquine, Ivermectin, Lopinavir/Ritonavir, Azith romycin, Zinc/Selenium
- Hydroxychloroquine, Ivermectin, Lopinavir/Ritonavir, Azith romycin, Zinc/Selenium
- Ivermectin, Lopinavir/Ritonavir, Azith romycin, Zinc/Selenium

Table 4 shows the mean comparison of direct bilirubin [DB] and total bilirubin [TB] of the experimental animals treated with different drugs. There was a significant difference in direct bilirubin of experimental animals treated with the drugs and combination 8 compared to control ($p<0.05$). Total bilirubin was significantly higher ($p<0.05$) in animals treated with ivermectin (0.93±0.10) and Lopinavir-ritonavir (0.92±0.06) when compared to control (0.47±0.07).

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Table 4: Showing the Results of Different Drug Effects on Direct Bilirubin Across the Various Studies Groups

Groups	Control	Chloroquine	Hydroxychloroquine	Ivermectin	Lopinavir-Ritonavir	Azithromycin	Zinc and Selenium	Combination 7	Combination 8	Combination 9	F Value	p-value
DB (mg/dL)	0.20±0.03	0.28±0.06	0.35±0.06	0.38±0.04	0.37±0.04	0.37±0.07	0.32±0.14	0.20±0.04	0.20±0.04	0.35±0.03	1.45	0.193
TB (mg/dL)	0.47±0.07	0.72±0.09	0.83±0.09	0.93±0.10*	0.92±0.06*	0.82±0.11	0.57±0.06	0.53±0.11	0.55±0.08	0.77±0.08	3.85	0.001

➢ *Key:*

- p≤0.05 Significant; p ≥ 0.05- Not significant. * Represents significant difference from control.
- DB=Direct bilirubin.
- TB=Total bilirubin.
- Combination7=Chloroquine+Ivermectin+Lopinavir+Ritonavir+Azith romycin+zinc+Selenium.
- Combination8=Hydroxychloroquine+Ivermectin+Lopinavir+Ritonavir+Zinc+Selenium.
- Combination9=Ivermectin+Lopinavir+Ritonavir+Azith romycin+Zinc+Selenium.

Figure 6 presents a bar chart illustrating the direct bilirubin concentration across the various study groups, highlighting there was a significant difference in direct bilirubin levels across treated groups compared to control. Figure 7 presents a bar chart showing the total bilirubin concentration across the various study groups, highlighting there was a significant increase in total bilirubin levels in animals treated with Ivermectin and Lopinavir- ritonavir compared to the control group.

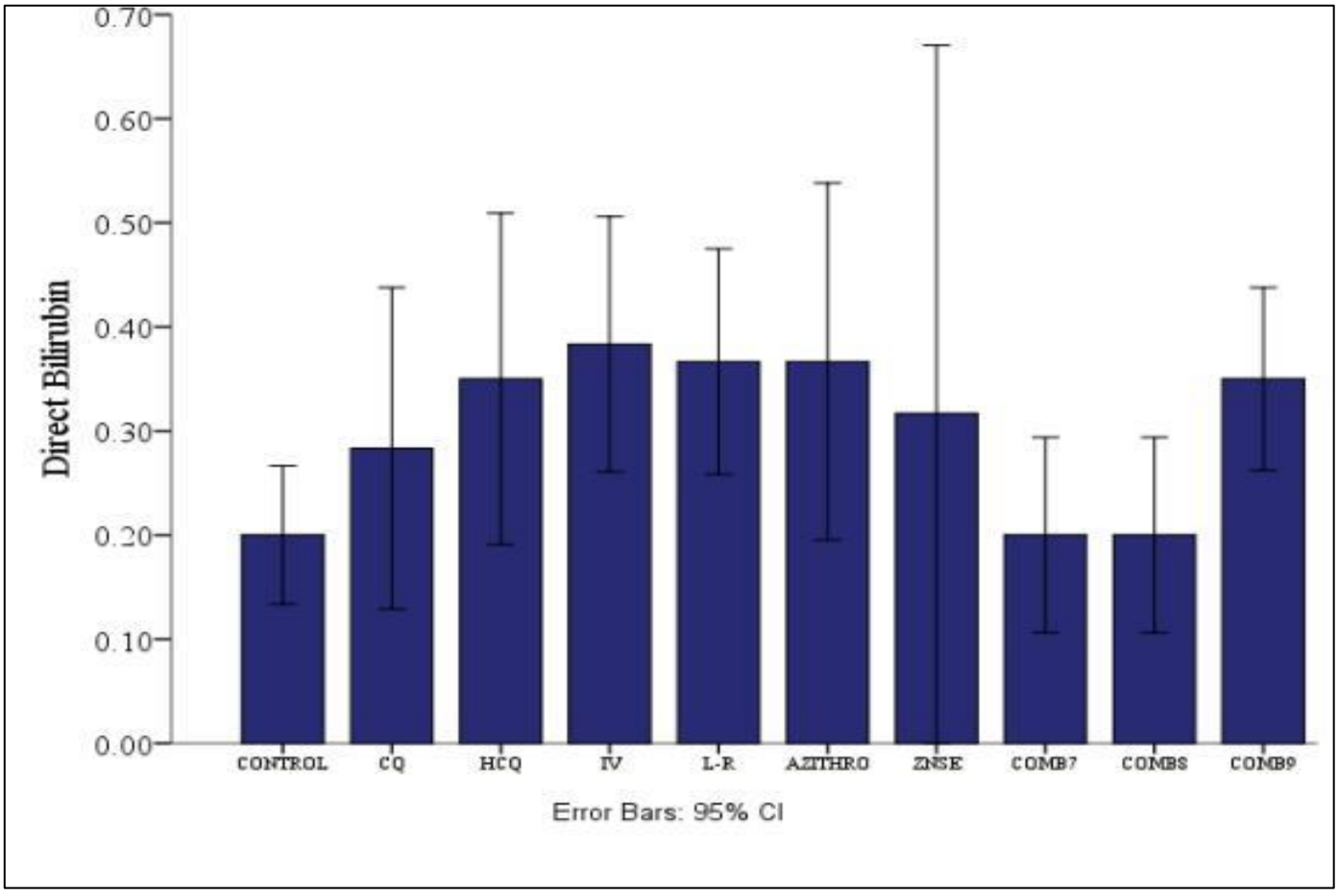

Fig 6: Showing the Direct Bilirubin Concentration Across the Various Studies Groups

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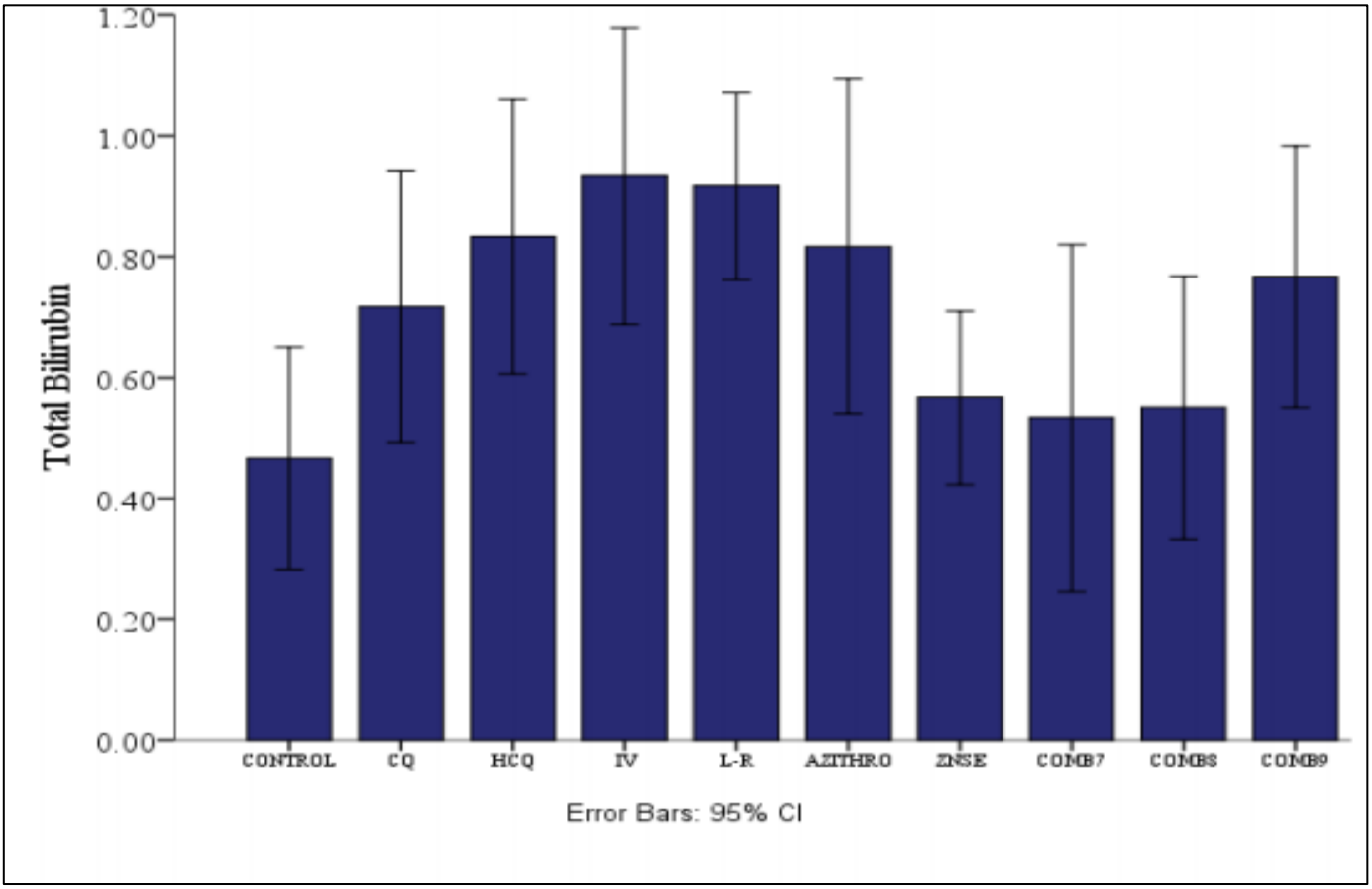

Fig 7: Showing the Total Bilirubin Concentration Across the Various Studies Groups

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CHAPTER FIVE
DISCUSSION, CONCLUSION AND RECOMMENDATION

A. Discussion

Glucose metabolism is a complex process essential for energy production and cellular function. It involves the uptake, utilization, and storage of glucose by various tissues, tightly regulated by hormones such as Insulin and Glucagon (Li et al., 2022). Glucose is converted into cellular energy through glycolysis, the citric acid cycle, and oxidative phosphorylation in the mitochondria. Dysregulation of glucose metabolism can lead to metabolic disorders like diabetes mellitus, characterized by abnormal blood glucose levels (Herold, 2023). Understanding the intricacies of glucose metabolism is crucial for maintaining metabolic homeostasis and developing effective interventions for metabolic diseases (Zhang et al., 2022).

The study observed a markedly elevated mean glucose level of 103.80 mg/dL ($P = 0.001$) in the HCQ + AZI + L.R + IV + Zn + Se group. Hydroxychloroquine (HCQ), an antimalarial agent, has been implicated in adverse effects on glucose metabolism, including insulin resistance and impaired insulin secretion (Yusuf et al., 2023). Likewise, Azith romycin (AZI), a macrolide antibiotic, and Lopinavir/Ritonavir (L.R), protease inhibitors used in the management of HIV infection, have been associated with alterations in glucose metabolism, potentially inducing hyperglycemia (Chen et al., 2022; Tadesse et al., 2022) through various mechanisms such as inhibition of insulin secretion or interference with glucose utilization. Intravenous (IV) administration of medications may further exacerbate dysglycemia by bypassing gastrointestinal regulatory mechanisms. Additionally, the inclusion of Zinc (Zn) and Selenium (Se) in the regimen may modulate glucose metabolism through their roles in insulin signaling pathways. The concurrent administration of these agents likely potentiates their individual effects, culminating in the observed elevation of blood glucose levels. However, the precise synergistic mechanism driving this phenomenon is not clearly understood. This study revealed notable alterations in weight following treatment with Chloroquine (CQ), hydroxychloroquine (HQ), ivermectin (IV), lopinavir/ritonavir (LR), azith romycin (AZI), and zinc (ZN), with all treatments demonstrating statistically significant increases compared to baseline ($p < 0.05$). Additionally, the combined regimen comprising CQ, IV, LR, AZI, ZN, and selenium (SE) exhibited a substantial elevation in weight post-treatment ($p < 0.05$). Conversely, the combination therapy involving hydroxychloroquine, AZI, LR, IV, ZN, and SE did not yield a significant weight change ($p > 0.05$). Hydroxychloroquine and chloroquine, derivatives of quinine, are known to exert various metabolic effects, including alterations in glucose and lipid metabolism (Yusuf et al., 2023). Similarly, azith romycin, a macrolide antibiotic, has been linked to alterations in glucose metabolism, potentially inducing hyperglycemia. (Hocqueloux et al., 2023). lopinavir/ritonavir have been associated with metabolic complications, including dyslipidemia and insulin resistance.

Zinc, an essential micronutrient, plays a crucial role in numerous metabolic processes, including appetite regulation, insulin signaling, and lipid metabolism (Banaszak et al., 2021). Zinc deficiency has been associated with reduced appetite and impaired glucose tolerance. (Squizani et al., 2022).

Similarly, selenium, another essential micronutrient, is involved in various metabolic pathways, including thyroid hormone metabolism and antioxidant defense mechanisms. Selenium deficiency has been linked to alterations in energy metabolism and thyroid function. (G haedi et al., 2023).

The COVID-19 virus, mainly affects the respiratory system, resulting in symptoms such as fever, cough, exhaustion, and shortness of breath, with possible consequences for other organs such as the kidneys (Wang et al.,2020).

Due to the immune response causing kidney inflammation and damage, acute kidney injury (AKI) is a sudden renal failure or damage that has emerged as a serious consequence in severe COVID-19 cases. For the diagnosis of acute renal damage, urea and creatinine are useful indicators (Yarijani and Najaf i, 2021).

According to Yarijani and Najaf i (2021), there have been various issues linked to repurposed medications used in COVID-19 clinical trials, especially those that damage the Kidneys and increase the risk of acute kidney injury (AKI).

Based on comparison, the research findings indicated that there was no statistically significant variation in the average urea levels between the different treatments. This implies that the urea levels of the group to which the treatment(s) was delivered were not significantly affected by it. Similarly, the mean creatinine did not significantly differ between the treatments. When it comes to COVID-19-related acute renal injury, these metrics provide information about the kidney health of the patients and specifically raise that risk. Liao et al., (2022) findings, which demonstrated that hydroxychloroquine administration did not raise creatinine levels or increase the risk of AKI, are consistent with the lack of significant differences observed in the mean urea and creatinine levels of the group treated with both Chloroquine and hydroxychloroquine. The results of Haroun et al., (2023) show that there was a substantial increase in albino rats treated with ivermectin compared to those given normal saline. This contrasts with the findings of this study, which show no significant difference in the mean urea and creatinine levels of the group treated with Ivermectin. According to Shi et al., (2021) the results of a non-randomized control trial, adult COVID-19 patients had significantly higher creatinine levels following lopinavir/ritonavir administration. However, the group treated with lopinavir/ritonavir did not experience a significant increase in creatinine levels. This group's reduction in mean urea and

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creatinine levels in comparison to the hydroxychloroquine-treated group is consistent with findings from Brown et al., (2021) showing acute renal injury was more common with hydroxychloroquine than azith romycin (15% vs. 0%). According to Olczak-Pruc et al., (2022), COVID-19 patients treated with zinc did not exhibit a statistically significant difference in the occurrence of acute kidney injury. Similarly, Zeng et al., (2021) highlight the significance of selenium supplementation because severe COVID-19 illness is linked to selenium deficiency. These findings are consistent with the lack of significant increase in the mean and urea creatinine levels of the zinc and selenium-treated groups compared to other groups.

In comparison to other groups, mean urea and creatinine levels were lower in groups 7 and 8, which received a combination treatment, and in groups receiving zinc and selenium. These reductions were not statistically significant, but they did point to a potential nephroprotective impact.

The COVID-19 drugs undergo rigorous preclinical and clinical evaluation for hepatotoxicity, and unexpected adverse effects may emerge, necessitating ongoing surveillance and monitoring of liver proteins and other markers of hepatic function (Clinton etal., 2021).

Findings from this study revealed a significant increase in direct bilirubin levels across treated groups compared to the control. This suggests that the drugs administered had a notable effect on the liver's ability to conjugate bilirubin, indicating a relatively unstable liver function in this aspect. This finding agrees with the results of a study by Hannafy et al., (2020), which reported alterations in direct bilirubin levels in animals treated with certain COVID-19 drugs.

There was a significant increase in total bilirubin levels in animals treated with Ivermectin and Lopinavir-ritonavir compared to the control group. This finding is consistent with the results of a study conducted by Oth man et al., (2022), which demonstrated a significant increase after administration of ivermectin. The similarity in findings across different studies underscores the potential hepato-toxic effects of these drugs, highlighting the importance of monitoring total bilirubin levels in patients undergoing treatment for COVID-19.

B. Conclusion

Elevated total bilirubin levels may indicate liver dysfunction or impaired excretion of bilirubin, potentially leading to jaundice or other liver-related complications (Merriman and Peters, 2008). This suggests that these drugs could have adverse effects on liver function, particularly in bilirubin metabolism and excretion pathways. Markedly elevated mean glucose levels in the HCQ + AZI + L.R + IV + Zn + Se group could indicate hyperglycemia and acute diabetes mellitus.

From the findings of this study, it can be conclusively drawn that at normal doses chloroquine, hydroxychloroquine, ivermectin, lopinavir-ritonavir, azith romycin, zinc, and selenium had no significant renal toxic effect on the urea and creatinine levels of albino rats. Though zinc, and combination drugs of groups 7 and 8 suggest nephroprotective effects, it is inconclusive as the changes in marker levels were not significant.

The findings revealed significant alterations in total bilirubin levels, following drug administration. These findings revealed significant alterations in bilirubin levels which may indicate acute liver injury and liver dysfunction showing the importance of vigilant and serial monitoring of liver function during the use of COVID-19 drugs.

C. Recommendation

Based on these findings, it is recommended to avoid the concurrent use of this specific drug combination (hydroxychloroquine + azith romycin + lopinavir/ritonavir + ivermectin + zinc + selenium) unless the potential benefits outweigh the risks of hyperglycemia for individual patients. If this combination is deemed necessary, close monitoring of glucose levels should be implemented, and appropriate management strategies should be in place to mitigate these metabolic effects.

The research also shows safe renal profiles for all drugs used in the study but further research with larger sample sizes and diverse patient populations can provide more comprehensive insights into the renal effects of COVID-19 treatments, particularly Hydroxychloroquine and azith romycin which showed high urea levels.

The renal profile of drugs repurposed should be assessed and the criteria for the selection of drugs for COVID-19 treatment due to the possible effect of increasing the risk of AKI.

Further research should be carried out on zinc and selenium, and combination drugs of group 7 and 8 due to their suggesting hepatoprotective effects.

The supplementation of widely used COVID-19 treatments with zinc and selenium such as the chloroquine and zinc combination is supported.

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Healthcare providers should implement regular monitoring of liver function parameters, including albumin, total bilirubin, and total protein levels, in patients receiving COVID-19 drugs.

Healthcare providers should exercise caution when prescribing medications known to impact liver function and tailor treatment strategies based on individual patient factors.

Healthcare providers should encourage patients to report any adverse events or symptoms suggestive of liver injury during treatment with COVID-19 drugs. Patients should be educated about the potential hepatotoxic effects of COVID -19 drugs and the importance of adhering to recommended monitoring protocols

D. Limitation

- Small Sample Size: A relatively small sample size of 60 rats, split into 10 groups, was used in the investigation. The findings' general izability may be impacted by this small sample size, which also raises the possibility of Type II errors, in which genuine effects are missed because of insufficient statistical power.
- Animal Model: Rats were used as the experimental model in this study. Although animal models can offer significant insights, their ability to accurately replicate the intricacies of human physiology and pathology may be limited, which in turn restricts the extrapolation of findings to human populations.
- Single Endpoint Measurement: As indicators of renal function, the study focused solely on measuring urea and creatinine levels. More metrics, such as the glomerular filtration rate (GFR) and a histological examination of the renal tissue, could provide a more thorough assessment of renal function.
- Absence of Clinical Data: The study was conducted in an experimental setting using animal models, and clinical data from human subjects were not included. Therefore, the translational relevance of the findings to human patients remains uncertain.
- Statistical Significance: Although some patterns in the data hinted at possible kidney-damaging or kidney-protecting influences within certain groups, the absence of statistically significant findings emphasizes the importance of interpreting the results carefully and avoiding overconfident conclusions.
- Addressing these limitations in future studies will help bolster the validity and practical applicability of the findings related to how repurposed drugs influence renal function in the context of treating COVID-19, thus providing a more comprehensive grasp of their impacts.

E. Contribution to Knowledge

- Taking this drug combination; Hydroxychloroquine + Azith romycin + Lopinavir/Ritonavir + Ivermectin + Zinc + Selenium may induce or worsen diabetes mellitus.
- COVID-19 repurposed medications may cause impaired excretion of bilirubin potentially leading to jaundice. Furthermore, the accumulation of bilirubin in the body can cause Multi-organ damage such as the brain (bilirubin encephalopathy), Heart, Kidneys as well as other tissues of the body.
- Since taking this combination could cause drug-induced acute liver injury, the liver function will be affected. Such consequences will include loss of G luconeogenesis function which will cause accumulation of ketones in the blood, leading to ketosis, lactic acidosis, hypoglycemia, coma, and death. This is worse in patients with Von Gierke Disease (Glycogen Storage Disease Type-1).

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APPENDIX I

A. Assay Procedure

The assay procedure for glucose estimation using the oxidase-peroxidase method typically involves the following steps:

- Prepare the Reagent Solution:The reagent solution contains the necessary enzymes (glucose oxidase and peroxidase), ch romogenic substrate, and buffer components. The composition and concentrations of the reagents may vary depending on the specific assay kit or protocol used.
- Sample Preparation: Depending on the sample type (e.g., whole blood, plasma, or serum), appropriate pretreatment steps may be required, such as deproteinization or dilution, to ensure compatibility with the assay and remove potential interfering substances.
- Reaction Mixture: Mix the prepared sample with the reagent solution in a suitable reaction vessel (e.g., test tubes or microplate wells). The reagent solution provides the necessary enzymes and ch romogenic substrate for the enzymatic reactions to occur.
- Incubation: Incubate the reaction mixture at a specific temperature (typically 37°C) for a predetermined time, allowing the enzymatic reactions to proceed and the color development to occur.
- Measurement: After the incubation period, measure the absorbance or optical density (OD) of the reaction mixture at a specific wavelength (e.g., 505 nm for o-dianisidine, 510 nm for 4-aminoantipyrine, or 420 nm for ABTS) using a spectrophotometer or a microplate reader.
- Calibration and Quantification: Construct a calibration curve by measuring the absorbance of known glucose standards with different concentrations. This calibration curve is then used to determine the glucose concentration in the unknown samples based on their absorbance values.

B. Advantages and Consideration

The oxidase-peroxidase method for glucose estimation offers several advantages, including:

- High Specificity: The method is specific for glucose, as glucose oxidase has a high affinity and selectivity for glucose as a substrate.
- Sensitivity: The ch romogenic substrates used in the assay can provide high sensitivity, allowing for the detection of low glucose concentrations.
- Simplicity: The assay procedure is relatively simple and can be automated for high-throughput analysis.
- Adaptability: The method can be adapted for various sample types (e.g., blood, plasma, serum, urine) by incorporating appropriate sample preparation

C. Steps.

However, it is important to consider potential interfering factors that may affect the accuracy of the assay, such as the presence of reducing substances or compounds that can interact with the enzymes or ch romogenic substrates. Appropriate controls and validation steps should be included to ensure reliable results.

The oxidase-peroxidase method for glucose estimation is widely used in clinical laboratories, research settings, and diagnostic applications due to its eliability, sensitivity, and suitability for automated analysis. It provides a robust and efficient means of quantifying glucose levels in biological samples, contributing to the diagnosis and monitoring of metabolic disorders, such as diabetes, and other health conditions related to glucose dysregulation.

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APPENDIX II

- 100µl of urease reagent was pipetted into the sample test, standard, and blank tubes respectively
- 10µl of the sample was added to the tube marked sample test
- 10µl of the standard was added to the tube marked standard
- Distilled water was added to the tube marked blank
- The tubes were mixed and incubated at 37°C for 10mins
- 2000µl phenol reagent was added to all the tubes
- 2000µl hypochlorite solution was added to all the tubes
- The tubes were mixed and incubated at 37°C for 15mins. Absorbance was read at 550nm against a reagent blank.

➢ *Calculation:*

Urea concentration (mg/dl) = Absorbance of test X Concentration of standard (80mg/dl Absorbance of standard.

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APPENDIX III

- 1000µl of picnic acid was pipetted into test tubes labeled sample, standard and blank respectively
- 1000µl alkaline solution was added to all the tubes
- 100µl of the sample was added to the sample test
- 100µl of the standard was added to the tube marked standard
- The tubes were mixed and incubated at room temperature for 10mins.

Absorbance was read at 500nm against the reagent blank.

- *Calculation:*
  Creatinine Conc. (mg/dl) = Absorbance of test X Standard Conc. Absorbance of standard

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EDO STATE
MINISTRY OF HEALTH

PROTOCOL NUMBER	**HA/737/24/C/0708320 (PLEASE QUOTE IN ALL ENQUIRIES)**
TITLE OF RESEARCH PROPOSAL	**EVALUATION OF THE IMPACT OF REPURPOSED COVID-19 MEDICATIONS ON SOME RENAL AND LIVER BIOMARKERS IN WISTAR RATS**
PRINCIPAL INVESTIGATOR (S)	**OBASUYI GRACE ELEOJO**
DATE CONSIDERED	**31ST JULY, 2024**
DECISION OF THE COMMITTEE	**APPROVED**

THIS APPROVAL DATES 31/07/2024 TO 31/07/2025. IF THERE IS A DELAY IN STARTING THE RESEARCH, PLEASE INFORM THE HREC EDO SMoH SO THAT THE DATES OF APPROVAL CAN BE ADJUSTED ACCORDINGLY

REMARK: Please kindly note that the HREC Edo SMoH seal authenticates this approval

DR (MRS) Omonyemen B. BELLO (MBBS, MPH, FPHCM) *(CHAIRMAN)* **SIGNATURE & DATE**..... 31/7/24

SUPERVISOR(S) 31/07/24

.....

ATTESTATION BY INVESTIGATOR(S)

No participant accrual or activity related to this research may be conducted outside of the approval dates. All informed consent forms used in this study must carry the Edo SMoH HREC-assigned number and duration of your research. No changes are permitted in the research without prior approval of the Edo SMoH HREC except in circumstances outlined in the Code. The Edo SMoH HREC reserves the right to conduct compliance visits to your research site without previous notification.

Signature & Date.....

State Secretariat Building, Block D - 2nd & 3rd Floors, P.M.B. 1113, Benin City, Edo State, Nigeria.