ADVERSE EFFECTS OF REPURPOSED COVID-19 DRUGS ON THE SERUM PROTEINS AND BILIRUBIN LEVELS IN WISTAR RATS

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BEING A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELORS DEGREE IN MEDICAL LABORATORY SCIENCE (BMLS) UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

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CERTIFICATION

This is to certify that this work carried out by **ANDY-OMEZI JESSICA** with matriculation number **BMS1802432**, is being submitted to the Department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin, Benin City, in partial fulfillment of the requirement for the award of Bachelor of Medical Laboratory Science degree.

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Head of Department

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EXTERNAL EXAMINER DATE

DEDICATION

I dedicate this project work to the Godhead, for his great faithfulness and commitment to all my affairs.

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I'm grateful to God for his strength, grace, and provision to go through the process.

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To the best mum in the world, I love you so much and your Ada made it! Sending all the hugs and kisses!

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ABSTRACT

The coronavirus disease (COVID-19) has presented a major threat to public health worldwide. COVID-19 is the result of infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that was first identified in Wuhan City, Hubei Province, China on December 2019. It is highly contagious and transmission is via respiratory droplets and direct contact. There are no specific antiviral measures available to treat COVID-19 but there are several treatment options that could be pursued as first-line therapy for COVID-19 which is the repurposing of drugs like Chloroquine, hydroxychloroquine, azithromycin, zinc, selenium, lopinavir/ritonavir and ivermectin. The aim of this project was to evaluate and monitor the adverse effects of the recommended drugs for the treatment of COVID 19 in the liver Proteins of Wistar rats. 60 rats were used for this study and the parameters that was assayed for was albumin, total protein, direct bilirubin and total bilirubin. Albumin was analysed using bromocresol green reagent, total protein was analysed using biuret reagent, and bilirubin by Evelyn and Malloy's method. The data generated were analyzed using Statistical Package for Social Sciences (SPSS) version 25.0 (IBM Inc. USA). The results showed that the Albumin of animals treated with Combination 7(2.93±0.14), Combination 8 (3.10±0.15) and combination 9 (3.08±0.15) were significantly lower than that of the control (4.17±0.18) ($p<0.05$). There was significant difference in direct bilirubin of experimental animals across most treated groups ($p<0.05$). It 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), and Total protein was significantly higher ($p<0.05$) in animals treated with ivermectin (8.62±0.45) when compared to control (7.02±0.22). In conclusion, the administration of these drugs adversely affected the synthetic and excretory functions of the liver. Regular assessment of liver function parameters, including albumin, total bilirubin, and total protein levels should be made compulsory in patients receiving COVID-19 drugs.

CHAPTER ONE

INTRODUCTION

1.1 Background of study

In December 2019, a new type of pneumonia with an unknown cause emerged in Wuhan, Hubei Province, China. Most of these initial cases were linked to the Huanan Seafood Wholesale Market. Through laboratory testing of fluid samples taken from the lungs of patients with this mysterious pneumonia, researchers were able to isolate and identify a novel coronavirus, later named SARS-CoV-2 (initially called 2019-nCoV). This previously unknown coronavirus was found to be the causative agent behind the outbreak of the disease termed COVID-19. The global spread of COVID-19, caused by the SARS-CoV-2 virus, rapidly escalated into a worldwide public health crisis of monumental proportions. The first cases of this viral respiratory illness were detected in individuals exposed at a seafood market in Wuhan in late 2019, as reported in a study by Zhu et al. (2020). After the COVID-19 outbreak began in late December 2019, the SARS-CoV-2 virus rapidly spread across the globe, reaching every continent. By March 18, 2020, the World Health Organization reported 179,111 confirmed cases and 7,426 deaths worldwide due to this viral disease. While primarily impacting the respiratory system, SARS-CoV-2 also attacked various other organs and bodily systems, leading to issues like heart damage, acute coronary events, kidney injury, gastrointestinal problems, and liver impairment (Guan *et al.,* 2020). In the early stages of the pandemic, healthcare providers explored repurposing existing drugs like chloroquine, hydroxychloroquine, and antivirals lopinavir/ritonavir as potential COVID-19 treatments based on their perceived efficacy against SARS-CoV-2. As research progressed, other therapies such as azithromycin, ivermectin, and micronutrient

supplements like selenium and zinc were also investigated as possible therapeutic options for managing this novel viral illness. Over time, the understanding of effective treatment regimens for COVID-19 rapidly evolved as medical professionals and scientists raced to find ways to combat the relentless global spread of the SARS-CoV-2 virus.

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).

1.2 Statement of problem.

SARS-COV 19 has propelled and driven the use of various medications for therapy, some of which are repurposed from already existing drugs. While these prescriptions can be a breakthrough in the alleviation of the disease, they can also have inadvertent and devastating consequences, one of which is the potential impact on the liver (Wang *et al*., 2020) a vital organ responsible for metabolism of endogenous and exogenous substances in the human body. Changes in the liver protein levels and activity can be an indication to drug induced liver injury (DILI) (Björnsson *et al.,* 2003). Having analysed the aftermath of the effects of these drugs on the liver, it would be in order to ask, what should be done in order to reduce the risks of DILI on patients? And how can the on going therapeutic management for the disease be improved upon for better results?

1.3 Justification of study

Some medications previously used to treat a variety of other diseases e.g antivirals, antimalarials, antimicrobials etc have been used for the therapeutic alleviation of COVID-19, keeping in mind

that the individual drugs had their existing uses and pharmacological purpose e.g Azithromycin for bacterial infection, chloroquine for anti malarial purposes and are now being used for the treatment of COVID-19. 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 intrest to liver proteins.

1.4 Aim

To evaluate the biochemical changes in the synthetic and excretory functions of the liver of Wister rats after the administration of recommended Covid-19 drugs.

1.5 Objectives

1) To access the harmful effects of Covid-19 drugs on total proteins.

2) To evaluate the toxic effects of the recommended drugs on the albumin synthesis.

3) To estimate the harmful outcomes of the drugs on total bilirubin and direct bilirubin levels.

1.6 Research Questions

1) Which of the COVID-19 drugs is most likely to affect liver proteins and the conjugation of bilirubin?

2) What specific liver proteins are impacted by these drugs and their combination?

3) By what mechanism does these drugs affect the liver proteins and bilirubin conjugation?

1.7 Null hypothesis

There is no statistically significant difference in liver protein levels between individuals who receive covid-19 drugs and those who do not.

1.8 Alternative hypothesis

There is a statistically significant difference in liver protein levels between individuals who receive COVID-19 drugs and those who do not.

CHAPTER TWO

LITERATURE REVIEW

2.1 Coronavirus

Coronaviruses are enveloped, positive-sense single-stranded RNA viruses belonging to the family Coronaviridae, which is further subdivided into four genera: Alpha-, Beta-, Gamma-, and Deltacoronavirus. To date, seven human coronaviruses (HcoVs) have been identified, classified under the Alpha- and Betacoronavirus genera. The Alphacoronavirus genus encompasses HcoV-NL63 and HcoV-229E, while the Betacoronavirus genus comprises HcoV-OC43, HcoV-HKU1, SARS-CoV (severe acute respiratory syndrome coronavirus), MERS-CoV (Middle East respiratory syndrome-related coronavirus), and the novel SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) (Zaki *et al.,* 2012). While alphacoronaviruses (HcoV-NL63 and HcoV-229E) and betacoronaviruses (HcoV-OC43 and HcoV-HKU1) are known to cause common colds, they can also lead to severe lower respiratory tract infections, particularly in vulnerable

populations such as the elderly and children (Weavers *et al.,* 2009). Specifically, HcoV-NL63 infection has been significantly associated with croup (laryngotracheitis) (Choi *et al.,* 2012), and HcoV-OC43 infection has been linked to severe lower respiratory tract infections in children (Zhu Yu *et al.,* 2018). In contrast, SARS-CoV and MERS-CoV are zoonotic coronaviruses capable of causing severe respiratory syndromes with catastrophic outcomes in approximately 90% of cases (Cui *et al.,* 2019).

2.2 Epidemiology

2.2.1. Source Of Infection

The infectious sources of SARS-CoV-2 encompass animal reservoirs and humans who have contracted the virus. Bats are considered the most probable initial hosts of SARS-CoV-2, while pangolins may have served as intermediate hosts. Notably, both symptomatic and asymptomatic individuals are capable of transmitting the virus (Huang *et al.,* 2020). However, the duration of viral shedding and the potential disruption of transmissibility during the natural course of the disease remain unclear (Huang *et al.,* 2020). Bats are the natural hosts for numerous known coronaviruses (de Wit *et al.,* 2016). As mentioned earlier, SARS-CoV-2 is a betacoronavirus, and infected humans are currently the primary sources driving the ongoing transmission. Asymptomatic patients, in particular, represent an unpredictable and insidious source of transmission that can go undetected. The unknown number of asymptomatic infections may explain why SARS-CoV-2 appears to be more contagious and widespread compared to SARS-CoV, whose transmission was largely limited to symptomatic patient sources (Huang *et al.,* 2020). The transmission potential of asymptomatic patients has been supported by a recent study that revealed indistinguishable dynamics of virus shedding between asymptomatic and

symptomatic individuals. Additionally, it has been demonstrated that higher viral loads are characteristic of the early stage of the disease and are more readily detectable in nasopharyngeal swab specimens than in oropharyngeal swabs (Zou *et al.,* 2020). Currently, the duration of transmission capacity remains uncertain, but two independent studies reported that infected individuals can transmit the virus during both the incubation period (Xu *et al.,*2020) and the recovery phase (Rothe *et al.,*2020).

2.2.2. Routes of transmission

Respiratory droplets are considered the primary mode of transmission for SARS-CoV-2, similar to other respiratory viral infections. The virus can spread when susceptible individuals come into contact with body fluids (such as sputum, saliva, or feces) containing the virus, either from humans or animals, through entry points like the oral cavity, nasal cavity, or other mucous membranes. Additionally, indirect transmission can occur when vulnerable individuals come into contact with contaminated surfaces or objects (*Wax et al.,* 2020). Biological aerosols refer to droplets containing pathogens (viruses or bacteria) suspended in the air for an extended period, forming droplet nuclei capable of spreading over longer distances through air currents, potentially leading to long-range disease transmission. Patients with severe SARS-CoV-2 infections may shed higher viral loads during certain medical procedures like mask ventilation, non-invasive ventilation, and tracheal intubation, generating localized aerosols that increase the risk of transmission to those in close proximity (Wax *et al.,* 2020). On February 6, 2020, Wuhan Tongji Hospital reported a case of a pregnant woman infected with SARS-CoV-2 who gave birth to a newborn testing positive for the virus 36 hours after delivery, suggesting the possibility of mother-to-child transmission. A recent study further revealed that fetal infection could occur in late pregnancy (Chen *et al.,*2020). These observations may be linked to the low expression of

ACE2 receptors in cells at the maternal-fetal interface (Zheng *et al.,* 2020). Overall, however, the risk of fetal infection through known vertical transmission routes appears to be minimal (Chen *et al.,*2020; Zheng *et al.,* 2020).

2.2.3 Incubation period

The knowledge of the incubation period of SARS-CoV-2 infection is key for implementing control measures and management. The estimated median incubation period is 5.1 days and 97.5% of the infected subjects will develop symptoms within 11.5 days of infection. These estimations suggest that after 14 days of observation or isolation, 101 out of 10,000 patients will likely show symptoms. (Lauer *et al.,* 2020). These estimations agree with findings from earlier research that found an incubation period ranging from 2.1 to 11.1 days, with a mean of 6.4 days (95% credible interval: 5.6–7.7). Thus, 14-day monitoring is recommended following contact with a probable or confirmed SARS-CoV-2 case (Algorithm for the management of contacts of probable or confirmed COVID-19 cases, 2020).

2.2.4 Molecular Diagnosis of SARS-CoV-2

Confirmation of cases with suspected SARS-CoV-2 infection is performed by detection of the unique viral sequences with nucleic acid amplification tests such as reverse real-time PCR (rRT-PCR). in a specimen collected from the upper respiratory tract (nasopharyngeal and oropharyngeal swabs) and if possible, from the lower respiratory tract (sputum, tracheal aspirate, or bronchoalveolar lavage) (Xie *et al.,* 2020).

2.2.5 Symptoms of SARS-CoV-2

Every age group is vulnerable to infection, including neonates and pregnant women. Most patients present with mild to moderate symptoms. The most common symptoms are fever, dry cough, fatigue; upper respiratory tract symptoms can include pharyngalgia, headaches, and myalgia. There is also one report describing patients with gastrointestinal symptoms, including abdominal pain and diarrhea in children and adolescents (Xu *et al*., 2020). In addition, asymptomatic patients have also been reported, although the frequency of this condition has not yet been determined. Approximately 20% of COVID-19 patients develop severe respiratory illness, with an overall case-fatality rate of about 2.3%. Patients with severe disease typically present with fever, dry cough, dyspnea, and bilateral pulmonary infiltrates on chest imaging. Complications of COVID-19 include ARDS, respiratory failure, liver injury, acute myocardial injury, acute kidney injury, septic shock, and even multiple organ failure. The risk factors for disease progression have not yet been established; however, preliminary evidence suggests that severe disease is more likely to take hold in individuals of older age, male sex, and in those with underlying co-morbidities. A study with 1099 confirmed COVID-19 patients was notable for the fact that about 23% had one or more underlying diseases, including chronic obstructive pulmonary disease (1.1%), hypertension (14.9%), diabetes (7.4%), coronary atherosclerotic heart disease (2.5%), and hepatitis B and liver cirrhosis (2.3%) (Guan *et al*., 2020).

2.2.6 Management

Currently, disease prevention and control, supportive care, and close monitoring are the essential key measures for population management of COVID-19. Severe or critically ill patients generally require oxygen therapy and intensive care as the disease frequently progresses to induce complications such as ARDS, respiratory failure, and septic shock. Despite all therapeutic

efforts, the mortality rate of patients in an intensive care unit (ICU) setting remains at about 40%. In efforts to reduce mortality related to severe COVID-19, attempts have been made to design therapies that either limit virus replication or modulate the host immune response.

2.2.7. Recommended drugs for the treatment of Covid-19.

In efforts to reduce mortality related to severe COVID-19, attempts have been made to design therapies that either limit virus replication or modulate the host immune response. Among these are treatment with Chloroquine, hydroxychloroquine, Ivermectin, Azithtromycin, Lopinavir / Ritonavir, Zinc/selenium and others.

2.2.7.1. Azithromycin

Azithromycin is classified as a macrolide antibiotic because of its unique ability (Imamura *et al*., 2005). AZM is actively absorbed by a variety of cells, including fibroblasts and white blood cells. (Rapp *et al*., 1998) AZM has immunomodulatory, anti-inflammatory, and antibacterial modulatory effects; hence, it is beneficial for patients with varying inflammatory diseases of the respiratory tract (Albert *et al*., 2011). It is also effective in patients with COVID-19 and has been used in clinical trials for the prevention of bacterial infection in these patients. It has been reported that AZM in combination with hydroxychloroquine (HCQ) can mitigate the viral load of SARS-CoV-2 (Gautret *et al*., 2020) Moreover, AZM can modulate the features of the immune system, that is, reducing cytokine production, maintaining epithelial cell integrity, and preventing lung fibrosis.

2.3.7.1.1 Pharmacology of Azithromycin

For bacteria to proliferate, they require a specific mechanism of protein synthesis facilitated by ribosomal proteins (Champney *et al.,* 1995). Azithromycin (AZM) inhibits bacterial protein synthesis by interfering with the transpeptidation/translocation step and preventing the assembly of the 50S ribosomal subunit, thereby controlling various bacterial infections. The strong affinity of macrolides, including azithromycin, for bacterial ribosomes is consistent with their broad-spectrum antibacterial activities (Dinos *et al.,* 2017). Azithromycin exhibits high stability in acidic environments, contributing to its extended serum half-life and increased tissue concentrations compared to erythromycin. Following oral administration, azithromycin has a bioavailability of 37%, with absorption unaffected by food. The serum protein binding of azithromycin varies in humans, decreasing from 51% at a concentration of 0.02 g/mL to 7% at 2 g/mL (Fohner *et al.,* 2017). Azithromycin undergoes minimal hepatic metabolism, with the majority of the drug being excreted unchanged through bile. The elimination of this drug primarily occurs via the liver (Singlas *et al.,* 1995).

2.2.7.2 Chloroquine

Treatment options for Chloroquine include infections of *P. vivax*, *P. malariae*, *P. ovale*, and susceptible strains of *P. falciparum*. It is also used off label for the treatment of rheumatic diseases, as well as treatment and prophylaxis of Zika virus (Shiryaev *et al*., 2017).

2.2.7.2.1 Pharmacology of Chloroquine

Chloroquine, a widely studied antimalarial drug, exerts its action by inhibiting the conversion of heme to hemazoin in malarial trophozoites, a crucial process facilitated by the enzyme heme polymerase (Coronado *et al.,*2014). As a result, Plasmodium species, the causative agents of

malaria, continue to accumulate toxic heme, ultimately leading to the death of the parasite. Chloroquine possesses the ability to passively diffuse through cell membranes and accumulate within endosomes, lysosomes, and Golgi vesicles. Once trapped within these organelles, the drug becomes protonated, leading to an increase in the surrounding pH (Wang *et al.,* 2020). This elevated pH within endosomes impairs the fusion and entry mechanisms of virus particles, thereby inhibiting their activity (Vincent *et al.,*2005). Chloroquine exhibits a large volume of distribution, ranging from 200 to 800 L/kg (Ducharme *et al.,* 2020), and is 46-74% bound to plasma proteins, with (-)-chloroquine displaying a stronger affinity for alpha-1-acid glycoprotein and (+)-chloroquine binding more strongly to serum albumin (Ofori-Adjei *et al.,*1996).

The metabolism of chloroquine is primarily mediated by the CYP2C8 and CYP3A4 enzymes, which catalyze N-dealkylation to form N-desethylchloroquine (Projean *et al.,* 2003). Subsequently, N-desethylchloroquine can undergo further N-dealkylation to yield N-bidesethylchloroquine, which is ultimately converted to 7-chloro-4-aminoquinoline through additional N-dealkylation steps. Chloroquine is predominantly eliminated through urinary excretion, with approximately 50% of the administered dose being recovered as unchanged chloroquine and 10% as desethylchloroquine in the urine (Ducharme *et al.,* 1996). The drug exhibits a half-life of Chloroquin and a total plasma clearance ranging from 0.35 to 1 L/h/kg.

2.2.7.3 Hydroxychloroquine

When there is no documented evidence of chloroquine resistance, hydroxychloroquine is used for the treatment of uncomplicated malaria (caused by *P. falciparum*, *P. malariae*, *P. ovale*, or *P. vivax*), chronic discoid lupus erythematosus, systemic lupus erythematosus, acute rheumatoid arthritis, and chronic rheumatoid arthritis.

2.2.7.3.1 Parmacology of Hydroxychloroquine

Research indicates that hydroxychloroquine has a multifaceted impact on the malaria parasite. Specifically, it accumulates in the parasite's lysosomes, altering the pH within the vacuole and disrupting the proteolytic breakdown of hemoglobin, crucial for the parasite's growth and replication (Fox, 1993). Moreover, hydroxychloroquine impedes the activity of parasite heme polymerase, causing the toxic accumulation of ferriprotoporphyrin IX (FP) (Chou *et al.,* 1992). This accumulation in human organelles also raises their pH, inhibiting antigen processing and presentation, thereby dampening the inflammatory response (Fox, 1993). Elevated pH levels may selectively affect the recycling of major histocompatibility complex (MHC) complexes, reducing the likelihood of autoimmune T cell activation. Furthermore, hydroxychloroquine suppresses cytokine release, such as interleukin-1 and tumor necrosis factor, potentially by inhibiting Toll-like receptors (Chary *et al.,* 2020). Regarding pharmacokinetics, hydroxychloroquine demonstrates 67-74% bioavailability, with no significant difference between enantiomers (Furst, 1996). It exhibits extensive tissue distribution, with a large volume of distribution from both blood and plasma (FDA, 2023). Metabolically, it undergoes N-dealkylation primarily by CYP3A4, yielding active and inactive metabolites, with desethylhydroxychloroquine being the primary metabolite (FDA, 2023). Following chronic administration, it shows an absorption half-life of 3 to 4 hours and a terminal half-life of 40 to 50 days, with a clearance rate of 96mL/min and renal clearance accounting for 16% to 30% of unchanged drug (FDA, 2023).

2.2.7.4 Ivermectin

Ivermectins are derived from the semi-synthetic antiparasitic drug ivermectin, which belongs to a class of highly-active broad-spectrum antiparasitic agents originally isolated from Streptomyces

avermitilis fermentation products. Its primary use in humans is for treating onchocerciasis, though it may also combat other worm infestations like strongyloidiasis, ascariasis, trichuriasis, and enterobiasis. Additionally, topical application is effective against head lice infestation. Notably, ivermectin exhibits broad-spectrum antiviral properties in vitro against RNA and DNA viruses, including HIV-1, dengue virus, influenza, Venezuelan equine encephalitis virus (VEEV), and Zika virus (Wagstaff *et al.,* 2012; Mastrangelo *et al.,* 2012; Pandey *et al.,* 2020; Yang *et al.,* 2020). In cell culture, a single dose of ivermectin effectively eliminated SARS-CoV-2 viral RNA within 48 hours, suggesting its potential use in treating COVID-19 patients during the early phase with mild to moderate symptoms.

2.2.7.4.1 Pharmacology of Ivermectin

Ivermectin binds selectively and with high affinity to glutamate-gated chloride ion channels in invertebrate muscle and nerve cells of the microfilaria. This binding causes an increase in the permeability of the cell membrane to chloride ions and results in hyperpolarization of the cell, leading to paralysis and death of the parasite (FDA, 2023). It is moderately well absorbed. Improved absorption with high fat meal. TheThe volume of distribution is 3 to 3.5 L/kg and it does not cross the blood-brain barrier. It's protein binding capacity is 93% (Klotz *et al.,* 1988) and the mode of excretion is primarily hepatic. Ivermectin and/or its metabolites are excreted almost exclusively in the feces over an estimated 12 days, with less than 1 % of the administered dose excreted in the urine. Following oral administration, the half-life of ivermectin is approximately 18 hours (Edwards *et al.,* 1988).

2.2.7.5 Zinc and Selenium

Zinc can be used to treat and prevent zinc deficiency and its effects, which include impaired wound healing, severe diarrhea in children, and stunted growth. It is also utilized for boosting the immune system, treating the common cold and recurrent ear infections, as well as preventing lower respiratory tract infections (*Cousins et al.,* 1996).

2.2.7.5.1 Pharmacology of Zinc and Selenium

Zinc enters the small intestine via a carrier-mediated mechanism, and under normal physiological conditions, absorption processes typically do not saturate transport pathways, making it challenging to precisely determine the absorbed amount. When administered in aqueous solutions to fasting individuals, zinc is efficiently absorbed (at a rate of 60-70%); however, absorption from solid diets is less effective and varies widely based on zinc content and diet composition (Bernie *et al.,* 2011). Generally, human zinc absorption is considered to be around 33% on average. Zinc absorption depends on concentration and increases linearly with dietary zinc intake up to a maximum rate. Approximately 60-70% of circulating zinc is bound to albumin and is released from food as free ions during digestion. These ions may combine with endogenously secreted ligands before being transported into enterocytes in the duodenum and jejunum (Nazanin *et al.,* 2013). Specific transport proteins may facilitate zinc passage across cell membranes into the hepatic circulation, and with high intake, passive paracellular absorption may occur. Absorbed zinc is transported via the portal system into hepatic circulation, subsequently entering the systemic circulation and being distributed to various organs. Although serum zinc represents only a small fraction of the body's zinc (0.1%), circulating zinc rapidly turns over to meet tissue demands. Gastrointestinal excretion accounts for about half of all zinc elimination, with significant amounts secreted through biliary and intestinal secretions, most of

which is reabsorbed, crucial for maintaining zinc balance. Other routes of zinc excretion include urine and surface losses (such as sloughed skin, hair, and sweat) (Tubek, 2007). The half-life of zinc in humans is approximately 280 days, with a clearance rate of 0.63 ± 0.39 μg/min observed in healthy individuals (Zinc and its importance for human health, 2013).

2.2.7.5 Lopinavir and Ritonavir

Lopinavir is an antiretroviral protease inhibitor used in combination with other antiretrovirals in the treatment of HIV-1 infection. Lopinavir is sold and administered exclusively in combination with ritonavir. This combination is needed due to lopinavir's poor oral bioavailability and extensive biotransformation. Ritonavir is a potent inhibitor of the enzymes responsible for lopinavir metabolism, and its co-administration "boosts" lopinavir exposure and improves antiviral activity (FDA, 2019).

2.2.7.5.1 Pharmacology of Lopinavir and Ritonavir

The HIV lifecycle comprises assembly, budding, and maturation, with the Gag polyprotein playing a central role in coordinating these stages as the primary structural proteins of the virus. The HIV-1 protease enzyme cleaves the Gag polyprotein, thus influencing various aspects of the HIV viral lifecycle. Lopinavir inhibits the HIV-1 protease enzyme, leading to the production of immature, non-infectious viral particles by preventing the proteolysis of the Gag polyprotein (Sundquist *et al.,* 2012). Due to its notably low oral bioavailability when administered alone (around 25%), lopinavir is exclusively co-administered with ritonavir to enhance bioavailability, inhibit drug metabolism, and achieve therapeutic concentrations (Sham *et al.,* 1998). The volume of distribution of lopinavir post-oral administration is approximately 16.9 L, with over 98%

plasma protein binding, primarily to alpha-1-acid glycoprotein and albumin, with a higher affinity for alpha-1-acid glycoprotein (Health Canada Product Monograph, 2001). Fecal elimination is the primary route for lopinavir, with approximately 10.4 ± 2.3% excreted in urine and 82.6 ± 2.5% in feces following oral administration (FDA, 2019). About 2.2% and 19.8% of the administered dose appear as unchanged parent drug in urine and feces, respectively. The elimination half-life of lopinavir is 6.9 ± 2.2 hours, and the estimated apparent clearance post-oral administration is approximately 6-7 L/h (Niu *et al.,* 2018).

2.4. Liver Proteins

Blood proteins, also termed plasma proteins, are proteins present in blood plasma. They serve many different functions, including transport of lipids, hormones, vitamins and minerals in activity and functioning of the immune system. Other blood proteins act as enzymes, complement components, protease inhibitors or kinin precursors. Contrary to popular belief, haemoglobin is not a blood protein, as it is carried within red blood cells, rather than in the blood serum. Serum albumin accounts for 55% of blood proteins, is a major contributor to maintaining the oncotic pressure of plasma and assists, as a carrier, in the transport of lipids and steroid hormones. Globulins make up 38% of blood proteins and transport ions, hormones, and lipids assisting in immune function. Fibrinogen comprises 7% of blood proteins; conversion of fibrinogen to insoluble fibrin is essential for blood clotting. The remainder of the plasma proteins (1%) are regulatory proteins, such as enzymes, proenzymes, and hormones. The role of the liver in metabolism of body proteins is abundantly illustrated by many observations of clinical as well as research nature. This role may be roughly divided into the following phases; in providing simple precursors from the metabolic pool for synthesis of tissue protein, in the formation of hepatic proteins, in the synthesis of serum proteins, in the storage of proteins, in the catabolic

process of breaking down proteins to amino acids, in deamination of amino acids to form urea and additionally in other transformation processes (e.g., transamination) of amino acids (Amalzroo *et al.,* 2017).

2.4.1. Determination of total protein

The methods of measuring total protein content of biological fluids are Biuret method: under strongly alkaline conditions, Cu2+ ions form multivalent complexes with peptide bonds in proteins. Binding shifts the absorption spectrum of Cu2+ ions to shorter wavelengths, leading to a color change fom blue to violet that has been termed the biurets reaction. Binding of Cu2+ ions to the organic compound biuret yeilds a simillar colour change, hence the name. The absorbance change from the protein addition is measure spectrophotometrically at 540nm, and this serves as a relatively simple method for quantifying proteins. Historically, the biuret method was not considered to react oth amino acids and dipeptides, but absorbance changes occurs with some amino acids, with amino acid amides, and with dipeptides. The biuret action was considered to react equally according to the peptide content with all proteins and peptides longer than two amino acids, but subsequently, peptides containing proline were noted to have reduced reactivity (Hortin and Meilinger, 2005). As long as proteins are not extremely proline rich or do not have a very unusual composition, different proteins probably have similar reactivities with respect to peptide content as long as the peptide the biuret reaction is performed in the typical endpoint maner. Bilirubin, lipemia and other serum compounds can introduce slight interferance with serum protein measurement by biuret assays, and calibration with albumin results in a slight bias relative to a kjeldahl method (Chromy *et al.,* 2009). Biuret assays are also available but should be considered as a seperate category from equilibrum assays. Cu2+ ions complex with small molecules and assessible sites in proteins almost instantaneously; additional absorbance change

over time for the kinetic analysis probably depends on the rate of unfolidng of a protein and exposure to additional binding sites for Cu2+ ions under strongly alkaline conditions. Different protiens are likely to unfold at different rates. An advantage of the kjeldahl biuret assay is the decreased effect of low molecular weight compounds; they usually react before the measuring interval occurs (Hortin and Meilenger, 2005). Direct optical methods can also be applied as 225nm to 290nm have been used to monitor protein concentrations and is commonly applied to chromatographic separation of proteins. Absorbance at 280nm depends primarily on the tryptophan and tyrosine content of the protein. This technique work best for purified proteins with known absorptivity. For complex mixtures, accuracy and specificity suffer from variable content of tryptophan and tyrosine and from absorbance at low molecular weight compounds such as free amino acids, uric acid, and bilirubin.

At 200 to 225 nm. peptide bonds are chiefly responsible for UV absorbance (70% at 1205); specific absorption by proteins at these shorter wavelengths is 10 to 30 times greater than at 280 nm (Johnson, 2006). Many low molecular weight compoundssuch as urea also have absorbance at wavelengths below 220nm. Accurate measurement of proteins by this method may require removal of low molecular weight molecules before absorbance measurements are performed. Several other optical methods using infrared or Raman analysis of specimens offer methods for protien determination based on complex spectral analysis (Hosafei *et al.,* 2007).

2.4 Serum Albumin

Albumin is the most abundant circulating protein found in plasma. It represents half of the total protein content (3.5 g/dL to 5 g/dL) of plasma in healthy human patients. Albumin is synthesized by liver hepatocytes and rapidly excreted into the bloodstream at the rate of about 10 gm to 15 gm per day. Very little albumin is stored in the liver, and most of it gets rapidly excreted into the

bloodstream. In humans, serum albumin functions as a significant modulator of plasma oncotic pressure and transporter of endogenous and exogenous (i.e. drugs) ligands. In clinical medicine, serum albumin can be measured via standard serum laboratory testing, and this measure has been advocated as a marker for an individual patient's nutritional status (Yuwen *et al.,* 2017).

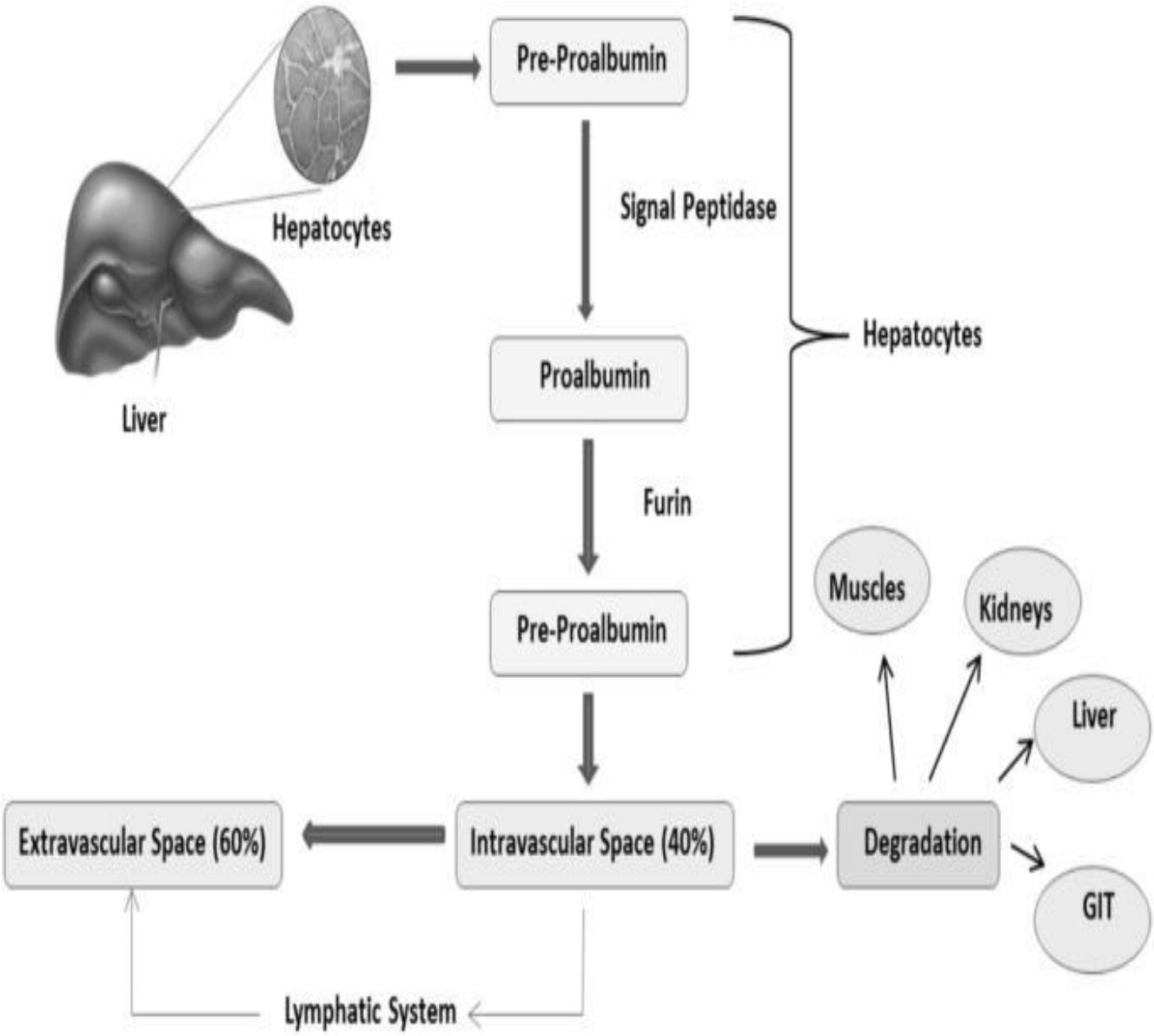

Fig 2.1 Albumin synthesis in the liver, Dewangan.(2020)

2.4.1 Albumin function and state due to liver changes.

Human albumin plays a crucial role in regulating plasma oncotic pressure and acts as a carrier for various substances known as ligands. These ligands transported by serum albumin encompass both endogenous, such as bilirubin, ions, and fatty acids, and exogenous, including drugs like methadone, propranolol, thiopental, furosemide, and warfarin, among others (Heuberger *et al.,* 2013). Hypoalbuminemia resulting from severe liver disease reduces available binding sites for exogenous drugs, potentially increasing drug sensitivity, particularly when serum albumin concentrations drop below 2.5 g/dL (Heuberger *et al.,* 2013). Upon entering circulation, approximately 30% to 40% of albumin remains in the bloodstream, while the rest enters the interstitial space, with the majority returning to circulation via the lymphatic system. Albumin's circulatory half-life is approximately 16 hours, and its osmotic effect primarily stems from its large molecular weight, with a lesser contribution from its negative charge, which attracts positively charged molecules and water into the intravascular compartment. By influencing oncotic pressure, albumin significantly impacts capillary membrane pressure and is exclusively synthesized in the liver. Experimental evidence, as confirmed by clinical observations, demonstrates a rapid decline in serum albumin levels post-hepatectomy and in hepatic diseases characterized by parenchymal destruction or loss (Sherlock *et al.,* 1975). However, in clinical settings, changes in serum albumin levels may occur slowly and may not immediately reflect acute liver damage. For instance, in acute viral hepatitis, serum albumin levels may initially be normal and decrease gradually only after parenchymal compromise occurs. Conversely, in liver diseases with minimal parenchymal involvement, such as biliary obstruction, serum albumin levels are typically normal. In severe and prolonged viral hepatitis and cirrhosis, serum albumin levels closely correlate with the clinical state and serve as prognostic indicators and for monitoring treatment outcomes. The essential role of hepatic parenchymal cells in albumin

synthesis is underscored by experimental studies in dogs exposed to prolonged ethanol administration, resulting in hepatic lesions akin to alcoholic liver disease in humans (Chey *et al.,* 1971).

2.5. 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 biliverdin, 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 (Capellini *et al.,* 2017). 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 (Dufour *et al.*, 2005). This condition may result from abnormalities at various stages of bilirubin metabolism, including excessive production, impaired liver uptake, conjugation defects, or defects in biliary 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).

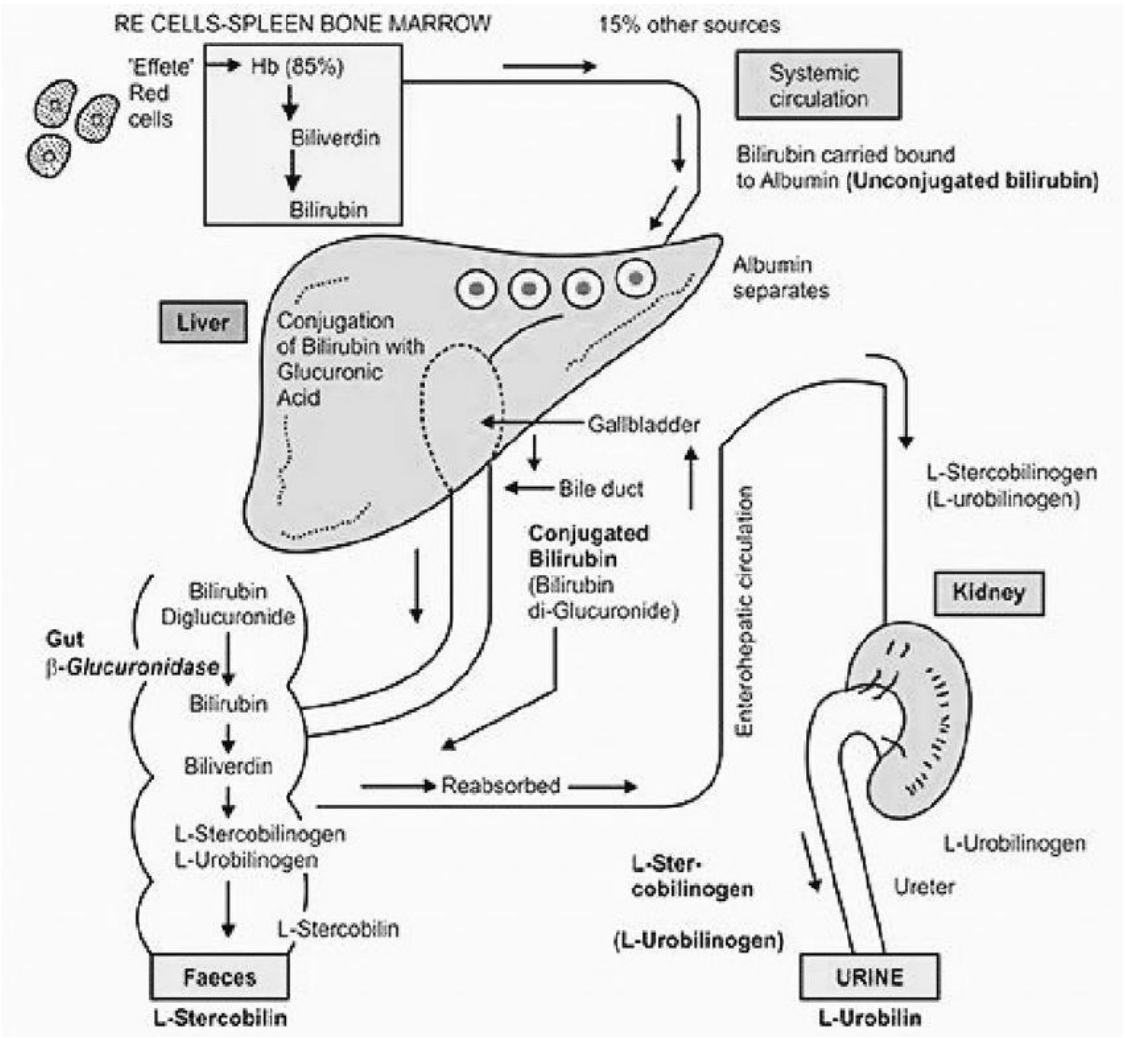

Fig 2.2 Bilirubin metabolism in the liver, Hamidreza. (2022)

2.5.1. Laboratory investigation of bilirubin

The reaction of bilirubin with the diazo reagent renders two color azodipyrroles (azopigments) that can be measured by spectrophotometry, at 530 nm to neutral or acid pH, and at 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 (Lo *et al.*, 2003). 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, it is crucial that minimum amounts of UCB react in the direct procedure. The diazo method described by Jendrassik & Grof in 1938 (Jendrassik, 1938) and later modified by Doumas et al. (Doumas, 1985) yields total serum bilirubin results which 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 (Perry *et al.,* 1983). Its trueness to measure total and direct bilirubin has been assessed by comparing with UCB and bilirubin diglucuronide quantified by nuclear magnetic resonance.

2.5.2 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 (Rutherford *et al.,* 2012). In this case, elevated levels of bilirubin in plasma are an indicator of poor prognosis and mortality (Eslami *et al.,* 2013). 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

(Helmke *et al.*, 2015). Bilirubin concentrations >17.6 mg/dL is an indication for hospitalization in patients with acute hepatitis unrelated to the intake of paracetamol. HepaticHepatic 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 (Feng *et al.*, 2018). The consequence is a neurotoxic effect, with progression to a higher level of encephalopathy due to increased concentrations of ammonium ion. High bilirubin concentrations are independent variables associated with the risk of 1-week mortality (López-Velázquez *et al*., 2014). Additionally, bilirubin concentrations ≥3.45 mg/dL in patients with chronic liver disease at hospital admission is a predictor of short-term mortality (Méndez-Sánchez *et al.,* 2017). Cholestatic liver diseases are characterized by bile flow suppression. Advanced disease causes increased bilirubinemia, generally conjugated (Helmke, 2015). 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 prothrombin 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).

2.6. 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 prothrombin 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 disease (Alqahtani *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).

Table 1: Changes in liver Proteins and enzymes in COVID-19 trials			
Drugs	**Toxicity**	**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 *et al., 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 *et al., 2020.*
Umifenovir	18.1%	Any adverse drug effect	Sun *et al., 2020.*
Remdesivir	15.2%	Liver injury	Kukarni *et al., 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	Grein *et al.,* 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 *et al.,* 2020

Table 1: Changes in liver Proteins and enzymes in COVID-19 trials			
Drugs	**Toxicity**	**Type of toxicity**	**Reference**
Azithromycin	1–2%	Elevation i,n serum aminotransferases	Her *et al.,* 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, upper limit of normal; CPT, Child Pugh Turcotte; ADEs, adverse drug events.

2.7 Drug combination 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 a 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).

2.8. 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 hazard from future administration and warrant prevention, or specific treatment, or alteration of the dosage regimen, or withdrawal of the product (Aronson *et al.,* 2005).

2.8.1. Types of ADRs

Drug reactions can be categorised as: type A which are dose-related reactions (adverse effects at either normal dose or overdose), eg. 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), eg. allergic or anaphylaxis reactions. Type C are dose and time-related reactions**,** eg due to dose accumulation, or with prolonged use (eg. adrenal suppression with corticosteroids). Type D are time related reactions**,** i.e. due to prolonged use in a drug which doesn't tend to accumulate (eg. tardive dyskinesia from antipsychotics), while type E are withdrawal reactions, i.e. the undesired effects of ceasing the drug (for example, opiate withdrawal). Type F reactions are as 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).

2.8.2 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 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 cholestatic, 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).

2.9 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 are 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 *et al.,* 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 stabilization of mast cells and basophils (Muraro *et al.,* 2014).

2.10 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).

CHAPTER TWO

MATERIALS AND METHODS

3.0. Study area

The study was carried out in the school of pharmacy, University of Benin, Benin City, Nigeria.

3.1. Materials

A mortar and pestle, volumetric flask, measuring cylinder, test tubes, surgical gloves, surgical dissecting kits, plain sample bottles, EDTA sample bottles, cotton wool, Bunsen flame, Ruler Heattich centrifuge (Rolotix 32A Germany), Analyser 1SE 4000 (SFR, France, Scout Pro digital balance, OHAUS corporation, USA), oral gastric tube.

3.2 Animals

Ethical approval was gotten from the ethics Committee of the Faculty of Pharmacy, University of Benin, Benin city, 9th-nov-2023. Albino rats weighing 100 to 200g were sourced from animal house at the Department of pharmacology and Toxicology, faculty of Pharmacy University of Benin, Benin city Nigeria. The rats were kept in plastic cages and allowed to acclimatize for two

weeks to adapt to the new environment in the plastic cage bedding with wood shavings under standard temperature (25ºc +_ 3ºc) and a 12:12hr natural light-dark cycle. The rats were fed with dry rodent pelletized finisher feeds and allowed free access to water. The bedding materials (wood shavings) of the cages was changed daily. All experiments were carried out in accordance with the National institute of Health Guidelines for the care and use of Laboratory Animals (NIA publications No 80 23 revised in 20).

3.3 Drugs and chemicals

Registered brands of the drugs (Chloroquine, Hydroxyl Chloroquine, Ivermectin, Lopinavir/Ritonavir, Azithromycin, Zinc/Selenium) from reputable manufacturers were obtained from a registered Pharmacy outlet. Calculated doses of each drug were dissolved in distilled water for oral administration according to the body weight of the rats using as oral gastric tube. Concentrated hydrochloric acid (HCL), sodium citrate, sodium bicarbonate and other needful chemicals were obtained from the departmental reagent store of the Department of Pharmaceutical Chemistry research laboratory.

3.4 Ethical Approval

Ethical approval with reference number **EC/FP/020/13** was sought and obtained from the Ethics Committee in the Faculty of Pharmacy, University of Benin, Edo State. A copy of the approval letter is attached at the appendix.

3.5 Experimental design

The male and female animals were dividend into six (6) groups of six animals per group, with each group containing three (3) male and three (3) female rats. And were treated orally with freshly prepared drugs (dissolved in measured volume of distilled water) for four (4) weeks as follows:

Table 3.1 The calculated therapeutic dose of administered Drugs

GROUP TREATMENT OPTION/COMBINATION

A (Negative control)	Six Rats were administered distilled water As Control.
B (Positive control)	Six rats were administered calculated therapeutic dose of Chloroquine dissolved in water.
C (Positive control)	Six rats were administered calculated therapeutic dose of Hydroxyl Chloroquine dissolved in water.
D (Positive control)	Six rats were administered calculated therapeutic dose of Ivermectin dissolved in water.
E (Positive control)	Six rats were administered calculated therapeutic dose of Lopinavir/Ritonavir dissolved in water.
F (Positive control)	Six rats were administered calculated therapeutic dose of Azithromycin dissolved in water
G (Positive control)	Six rats were administered calculated therapeutic dose of Zinc/Selenium dissolved in water.
H (Positive control)	Six rats were administered calculated combined therapeutic dose of Chloroquine, Ivermectin,Lopinavir/Ritonavir, Azithromycin, Zinc/Selenium dissolved in water.
I (Positive control)	Six rats were administered calculated combined therapeutic dose of Hydroxyl Chloroquine, Ivermectin, Lopinavir/Ritonavir, Azithromycin, Zinc/Selenium dissolved in water.
J (Positive control)	Six rats were administered calculated combined therapeutic dose of Ivermectin, Lopinavir/Ritonavir, Azithromycin, Zinc/Selenium dissolved in water.

3.6. Determination Of Change In Body Weight

The weights of the animals were taken before drug administration, 10 days after commencing experimental procedure and at the completion of the 3 weeks drug administration period using the Scout Pro digital balance (OHAUS corporation, USA) and the change in weight was determined.

3.7. Number of Rat Per Group

Total number of animals to be given Chloroquine= 6rats

Total number of animals be given Hydroxyl Chloroquine

= 6rats

Total number of animals be given Ivermectin = 6rats

Total number of animals be given Lopinavir/Ritonavir = 6rats

Total number of animals be given Azithromycin

= 6rats

Total number of animals be given Zinc/Selenium

= 6rats

Total number of animals be given Chloroquine, Ivermectin, Lopinavir/Ritonavir, Azithromycin, Zinc/Selenium = 6rats

Total number of animals be given Hydroxyl Chloroquine, Ivermectin, Lopinavir/Ritonavir, Azithromycin, Zinc/Selenium = 6rats

Total number of animals be given Ivermectin, Lopinavir/Ritonavir, Azithromycin, Zinc/Selenium =

6rats

3.8 Dosage calculations

Therapeutic dose calculation

Volume of 0.5mL of drug Administered

If 200g Rat = 0.5mL

Then 153g Rat is equivalent to 0.38mL

Or

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 Hydroxyl Chloroquine in cage 2

0ne 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

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 Azithromycin 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 = 0.3175ml

Therapeutic dose calculation for Zinc/Selenium in cage 6

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

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

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

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

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

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

Black faeces was observed in this cage day 13

Therapeutic dose calculation for Chloroquine, Ivermectin, Lopinavir/Ritonavir, Azithromycin & Zinc/Selenium (Combination 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 = 0.325ml

Therapeutic dose calculation for Hydroxyl Chloroquine, Ivermectin, Lopinavir/Ritonavir, Azithromycin & Zinc/Selenium (Combination 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, Azithromycin & Zinc/Selenium (Combination 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

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

Preparation and administration of drugs

Calculated standard doses was computed in kilogram/body weight. The tablets were grounded into powder and triturated using water. The solution was transferred into previously calibrated container and made up to volume. The final preparation was administered using orogastric tube into the albino rats grouped into six groups. They were administered the freshly prepared drugs for twenty-eight days. The animal was anaesthetized using chloroform and sacrificed. Blood samples collected and assayed for traditional toxicity profile as described (Aghahowa *et al*., 2023).

3.9 Sample collection

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 anaesthetized rat was dissected carefully. Then using a sterile 5 ml syringe with a 23G1 needle, about 2 ml of blood was withdrawn from the retro-orbital sinus vein and heart of each rat and transferred to a pre labelled lithium heparin (blue) tubes for liver enzymes analysis. Blood samples were first centrifuged at 40 rpm/minute for 3 minutes. The plasma (clear portion) was carefully collected into pre labelled plain (red color) tubes using different sterile syringes for each, and the serum discarded

3.10 Liver Indices

The common markers that were used to assess the fate of Chloroquine, Hydroxyl Chloroquine, Ivermectin, Lopinavir/Ritonavir, Azithromycin, Zinc/Selenium in the liver were aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, conjugated bilirubin, total bilirubin, albumin and total proteins. These were assessed using serum samples prepared from the rats.

3.10.1 Assay of Total proteins

Principle of test

Cupric ions in an alkaline medium interacts with protien peptide bonds resulting in the formation of a coloured complex that is measured photometrically.

Procedure

- The tubes for test, standard and blank was set up
- 20ul of the sample was added to the test
- 20ul of standard was added to the standard test tube
- 20ul of diluted water was added to the blank
- 1ml of biuret reagent was added to all the tubes
- The tubes were incubated at room temperature for 30mins and absorbance was read at 546nm.

Calculation

Total protein concentration = Absorbance of test/Absorbance of standard × concentration of standard (g/dL).

Reference range

6.0-8.3g/dl

3.10.2 Assay of Albumin

Principle of Test

The measurement of albumin is based on its quantitative binding to the indicator 3: 3. 5, 5°-tetrabromo-m cresol sulphophthlein (bromocresol green, BCG). The albumin- BCG- complex

absorbs maximally at 578mm, the absorbance being directly proportional to the concentration of albumin in the sample.

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

Calculation

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

Reference range

3.3-5.2g/dl

10.3. Assay of Bilirubin

Principle of Test

Bilirubin is determined in the presence of caffeine by the reaction with diazotized sulphanilic 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 test, standard and blank.
- 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

3.11 Quality Control

1. Analytical grade of reagents were used throughout the research process.

2. Reagents were checked for expiry date before use.

3. The equipment was calibrated properly

4. The stability of calibration was checked periodically.

5. Standards of various parameters were subjected through various test methods in order to check the reliability of the data.

3.12 Statistical Analysis

Data obtained from this research was presented and analyzed using Statistical Package for Social Sciences (SPSS) version 25.0 (IBM Inc. USA). Analysis of Variance (ANOVA) was used to compare the treatment groups and the results was expressed in means ± SEM and bar charts. Differences was considered to be significantly different when the p value obtained was < 0.05.

CHAPTER FOUR

RESULTS

Table 4.1 shows the mean comparison of red liver proteins of the experimental animals treated with different drugs. Albumin of animals treated with Combination 7 (2.93±0.14), combination 8 (3.10±0.15) and combination 9 (3.08±0.15) were significantly lower than that of the control (4.17±0.18) ($p<0.05$). 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). Total protein was significantly higher ($p<0.05$) in animals treated with ivermectin (8.62±0.45) when compared to control (7.02±0.22).

Groups	Control	Chloroquine	Hydroxychloroquine	Ivermectin	Lopinavir-Ritonavir	Azithromycin	Zinc and Selenium	Combination 7	Combination 8	Combination 9	F Value	p value
Albumin (g/dL)	4.17±0.18	4.33±0.11	4.67±0.18	4.67±0.11	4.82±0.11	5.08±0.32	3.42±0.34	2.93±0.14*	3.10±0.15*	3.08±0.15*	17.07	0.001
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
TP (g/dL)	7.02±0.22	6.97±0.26	7.10±0.19	8.62±0.45*	7.48±0.29	7.67±0.53	6.75±0.50	5.83±0.56	6.27±0.20	6.08±0.18	6.48	0.001

Key: p≤0.05 Significant; p ≥ 0.05- Not significant. * Represents significant difference from control, DB=Direct bilirubin.
TB=Total bilirubin.
TP=Total protein.
Combination7=chloroquine+ivermectin+lopinavir+ritonavir+azithromycin+zinc+selenium.
Combination8=hydroxychloroquine+ivermectin+lopinavir+ritonavir+zinc+selenium.
Combination9=ivermectin+lopinavir+ritonavir+azithromycin+zinc+selenium.

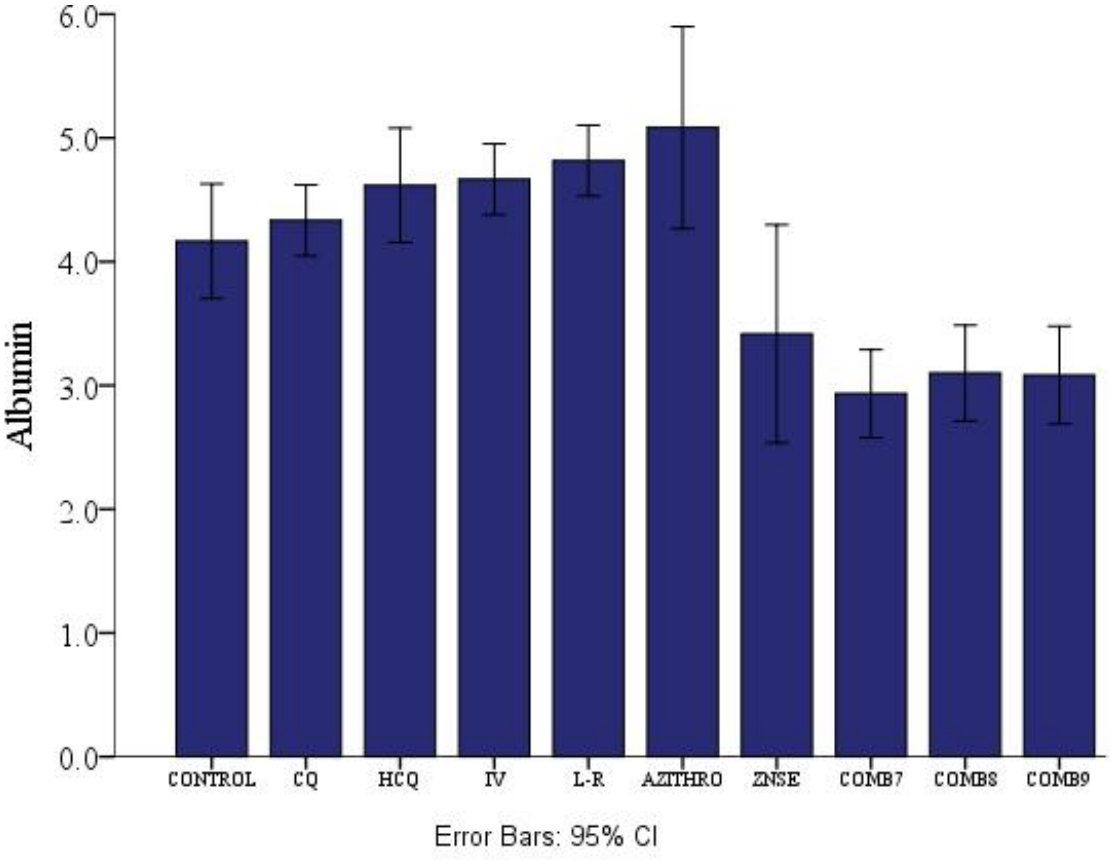

Figure 4.1. showing the albumin concentration across the various studies groups.

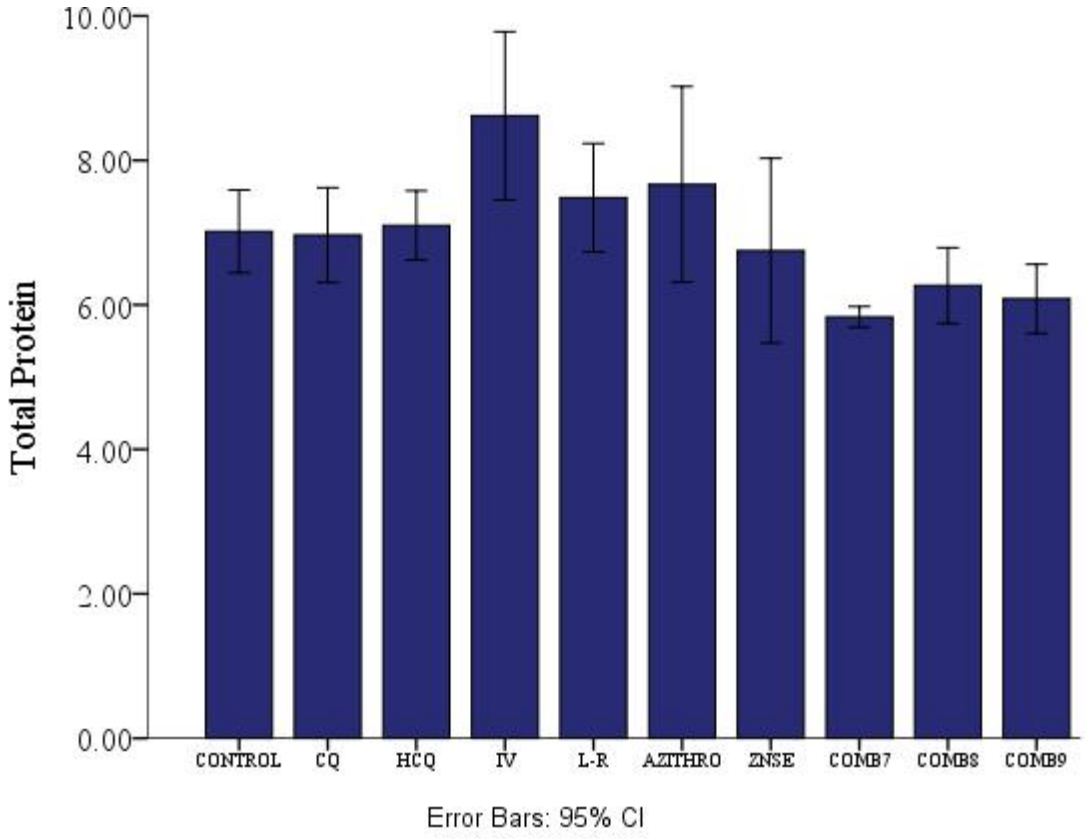

Figure 4.2. showing the total protein concentration across the various studies groups.

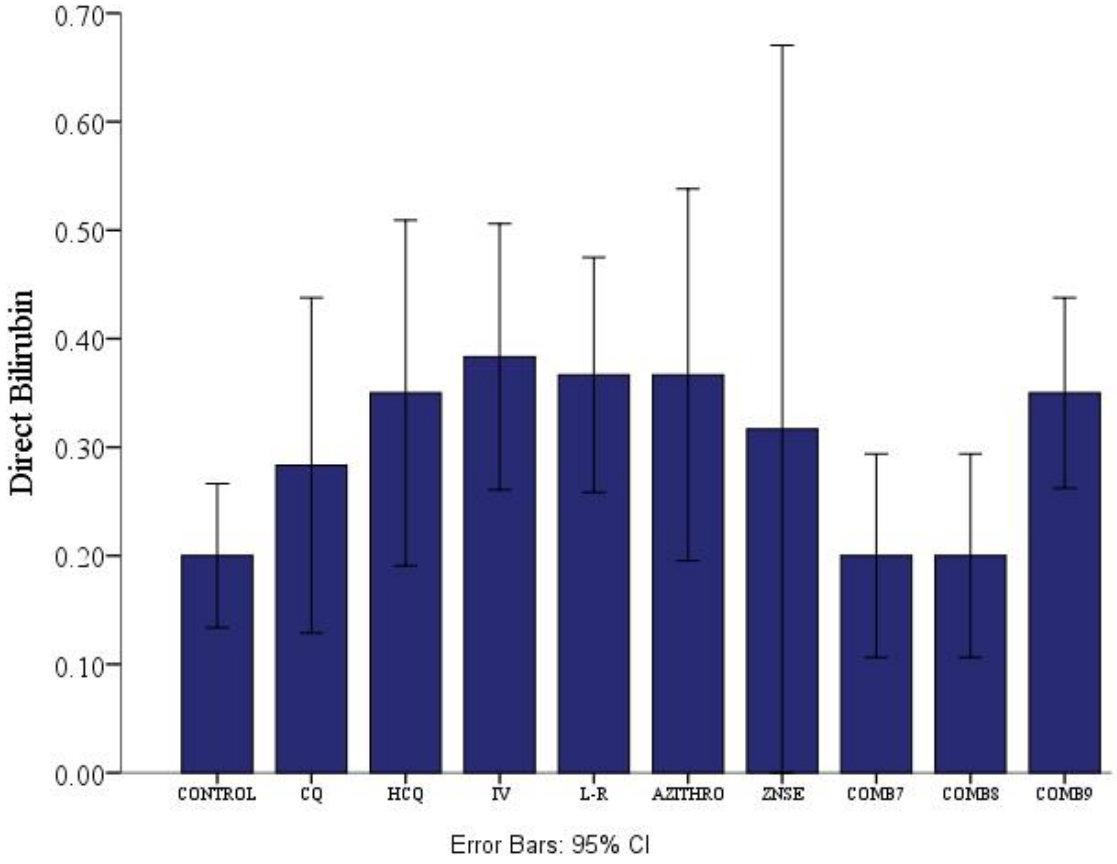

Figure 4.3. showing the direct bilirubin concentration across the various studies groups.

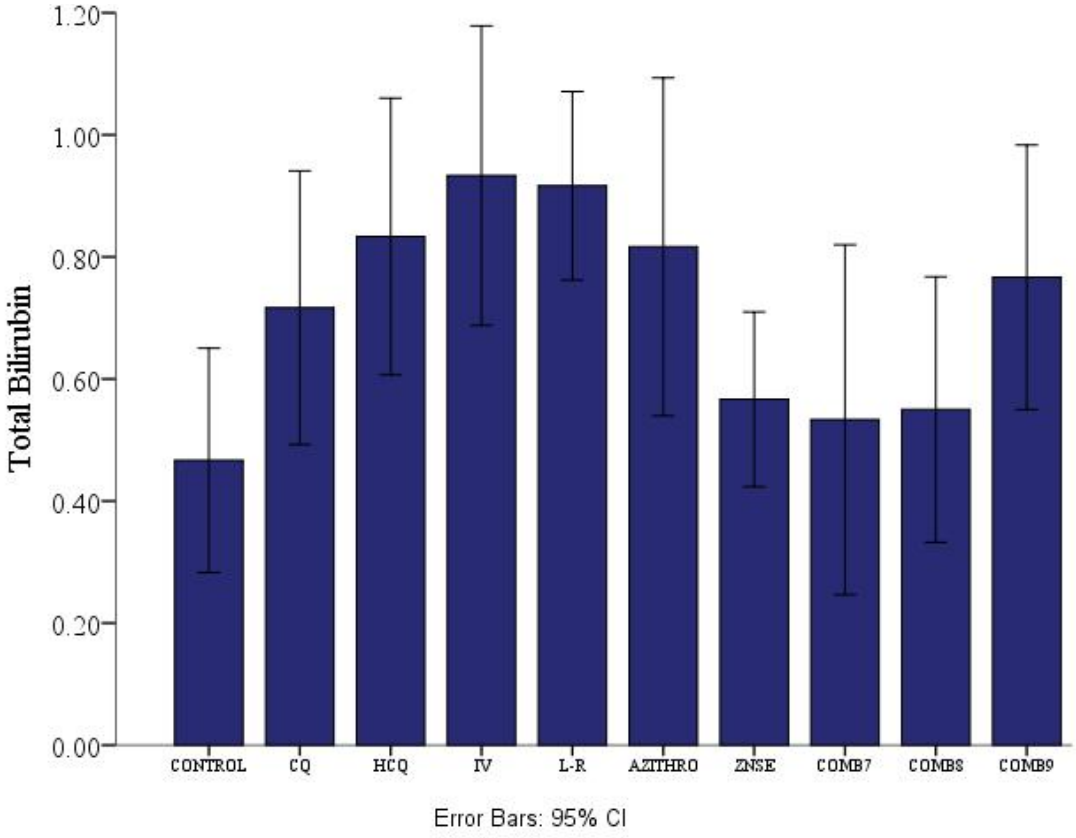

Figure 4.4. showing the total bilirubin concentration across the various studies groups.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1. DISCUSSION

The COVID-19 pandemic has brought about a global effort to develop therapeutics to combat the novel coronavirus, SARS-CoV-2. Among the drugs utilized in the treatment of COVID-19, a considerable focus has been directed towards antiviral agents, immunomodulators, and other pharmaceutical interventions (Lam *et al*., 2020). However, as with any pharmacological intervention, the potential for adverse effects and unintended consequences necessitates thorough investigation, particularly concerning hepatic function (Alomar, 2014).

The liver plays a crucial role in drug metabolism and detoxification, making it susceptible to the effects of various medications (Singh *et al*., 2016). Moreover, COVID-19 itself can induce hepatic injury through direct viral infection or secondary mechanisms, emphasizing the importance of understanding how COVID-19 drugs may impact liver function (Nardo *et al*., 2021). Given the significance of liver proteins in maintaining hepatic homeostasis and function, assessing the effects of COVID-19 drugs on these proteins is paramount. An understanding of the impact of COVID-19 drugs on liver proteins requires a thorough approach. First and foremost, it is essential to evaluate the direct effects of these drugs on the expression, activity, and stability of key liver proteins involved in various physiological processes. Furthermore, the potential for drug-induced liver injury must be carefully considered. While many COVID-19 drugs undergo rigorous preclinical and clinical evaluation for hepatotoxicity, unexpected adverse effects may emerge, necessitating ongoing surveillance and monitoring of liver proteins and other markers of hepatic function (Clinton *et al*., 2021).

Albumin, a major protein produced by the liver, plays a crucial role in maintaining colloidal osmotic pressure and transporting various substances in the blood (Hankins, 2016). The results from this study indicate a significant decrease in albumin levels in animals treated with Combination 7, Combination 8, and Combination 9 which has a combination of multiple drugs (chloroquine, hydroxychloroquine, ivermectin, lopinavir, ritonavir, azithromycin, zinc and selenium) compared to the control group. This is in contrast with previous research by Satsangi *et al*. (Satsangi *et al*., 2021), which reported mainly no changes in albumin levels following administration of similar drug combinations in animal models. The decreased albumin levels observed in our study suggest a potential disruption in liver function. This reduction suggests a potential disruption in liver function caused by these drug combinations. The decrease in albumin levels may affect the body's ability to maintain fluid balance and transport essential molecules, which could have implications for overall health (Hankins, 2006).

Findings from this study revealed significant difference 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 instable 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. 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. This finding is consistent with the results of a study conducted by Othman *et al*. (2022), which

demonstrated a significant increase after administration of ivermectin. The similarity in findings across different studies underscores the potential hepatotoxic effects of these drugs, highlighting the importance of monitoring total bilirubin levels in patients undergoing treatment for COVID-19. There was a significant increase in total protein levels in animals treated with Ivermectin compared to the control group. This elevation in total protein levels may reflect enhanced liver protein synthesis or altered protein metabolism due to drug administration. However, further investigation is needed to elucidate the underlying mechanisms and potential implications of this increase in total protein levels. This finding contrasts with previous research by Othman *et al*. (2022), which reported a significant decrease in total protein levels following administration of Ivermectin in animal models. The discrepancy between our findings and previous studies suggests that the effects of Ivermectin on total protein levels may vary depending on factors such as experimental conditions or animal models used. These findings suggest that the administration of certain COVID-19 drugs can lead to alterations in liver protein levels which emphasizes the importance of monitoring liver protein parameters during the use of COVID-19 drugs to mitigate potential adverse effects on liver health.

5.2. CONCLUSION

This study investigated the effect of various COVID-19 drugs on liver protein levels in experimental animals. The findings revealed significant alterations in liver protein parameters, including albumin, total bilirubin, and total protein levels, following drug administration. These findings show the importance of vigilant monitoring of liver function proteins during the use of 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.

5.3. RECOMMENDATIONS

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

2) Healthcare providers should encourage patients to report any adverse events or symptoms suggestive of liver injury during treatment with COVID-19 drugs.

3) Patients should be educated about the potential hepatotoxic effects of COVID-19 drugs and the importance of adhering to recommended monitoring protocols

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