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**Drug Discovery**

# Cancer Markers Induction Potential and Adverse Effects of Anti-COVID-19 Drugs in *Rattus norvegicus*

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## ABSTRACT

In recent times, COVID-19 infection co-existing with cancer had generated worrisome situation globally. This study assessed effects of COVID-19 combination drugs on prostate, ovarian, breast and liver cancer biomarkers in rats (*Rattus norvegicus*). Healthy rats were selected, and grouped as: Group 7 (chloroquine 10mg/kg, ivermectin 200mg/kg, zinc 2.5mg/kg, azithromycin 5mg/kg, lopinavir/ritonavir 4/1mg/kg, selenium 3.33mg/kg), Group 8 (hydroxychloroquine 6.5mg/kg, ivermectin 200mg/kg, lopinavir/ritonavir 4/1mg/kg, azithromycin 5mg/kg, zinc 2.5mg/kg, selenium 3.33mg/kg), Group 9 (ivermectin 200mg/kg, lopinavir/ritonavir 4/1mg/kg, azithromycin 5mg/kg, zinc 2.5mg/kg, selenium 3.33mg/kg). These combinations were administered orally for twenty-eight days. Blood samples were collected and assayed for common toxicity and cancer biomarkers after euthanization using chloroform. The combined drugs significantly altered body weight, glucose, alkaline phosphatase, conjugated bilirubin, total protein, albumin, high-density lipoprotein, white blood cells, lymphocytes %, granulocytes %, lymphocytes $\times 10^3 \mu l$, red blood cells, hemoglobin, hematocrit, mean cell volume, red cell distribution weight, platelets, platelet concentration transmittance ($P<0.05$). Cancer Antigen -125 ($P^*$ Vs 7, 8, 9) decreased significantly, while Alpha-Fetoprotein ($P^*$ Vs CON), Prostate Specific Antigen ($P^*$ Vs CON, 8), and Cancer Antigen 15-3 ($P^*$ Vs 7, 8, 9) increased significantly. These annotated results had shown alterations in some common toxicity and cancer biomarkers, therefore there is need for caution in the selection of drugs for COVID-19 infection treatment.

**Keywords**: COVID-19 infection, Drugs, Toxicities, Cancer markers.

DISCOVERY SCIENTIFIC SOCIETY

## 1. INTRODUCTION

The global scourge of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) that is commonly called COVID-19 (Branswell and Joseph, 2020; WHO, 2020) had ravaged some regions gloally in recent times. Chemotherapeutic agents recommended were hydroxychloroquine, interleukin one inhibitor (anakinra), interleukin six inhibitor (tocilizumab, sarilumab, siltuximab), chloroquine, lopinavir/ritonavir, remdesivir, azithromycin, interferons (Alfa, Beta), Janus Kinase Inhibitors (JAK) (Baricitinib), HMG-CoA Reductase Inhibitors (Statins), Non-steroidal Anti-Inflammatory Drugs (CDCP, 2019; Calina *et al*., 2020). These recommended drugs have different mechanisms of action and have been found helpful in the treatment of other diseases (Dockrell *et al*., 2010; Brunton *et al*., 2011; Rang *et al*., 2012; Baxter and Towers, 2017). In the trial efficacy against COVID-19 infection (Gralinski and Baric, 2015; John and Kearney, 2020; Wang *et al*., 2020a; Wang *et al*., 2020b), these agents have shown in *in vivo* and *in vitro* activities in the viral life cycle as reported (Fung *et al*., 2019; Shereen *et al*., 2020; John and Kearney, 2020). There are reported co-morbidities that had existed with COVID-19 infection (Wang *et al*., 2000a; Garg *et al*., 2020; Wu *et al*., 2020; Guan *et al*., 2020; Cai *et al*., 2020; CDCP, 2019). The most worrisome among these is cancer. There are also reports of identified tumor markers (Waxman, 1995; NCI, 2023), and standard methods of detection (Waxman, 1995; Lindblom and Liljegren, 2000, Schrohl *et al*., 2003; Sokoll and Chan, 2004; Cooper, 2004; Wu 2007). Interestingly, there are documented conventional therapies for different types of cancer in different regions (Atkins *et al*., 1999; Ciardiello and Tortora, 2008; Jordan, 2003; Hodi *et al*., 2010; Dowsett *et al*., 2010; Chapman *et al*., 2011; de Bono *et al*., 2011; Topalian *et al*., 2012; Smith *et al*., 2012; Baskar *et al*., 2012; Maude *et al*., 2014; Shaw *et al*., 2014; Kumar *et al*., 2016; Lord and Ashworth, 2017; June *et al*., 2014; Lamba *et al*., 2019; NCI, 2021). Since COVID-19 infection may co-exist with cancer, there may be need to select some of drugs recommended and use them as combinations. These drugs may worsen existing cancer pathologies in raising the biomarkers. The study therefore assessed the toxicity pattern and possible prostate, ovarian, breast, and liver cancer markers induction/inhibition potential in rats (*Rattus norvegicus*) of some recommended drugs as a combination for the treatment of COVID-19 infection.

## 2. MATERIALS AND METHODS

The study was carried out in the Department of Pharmacology and Toxicology, University of Benin, Nigeria and the Department of Chemical Pathology, University of Benin Teaching Hospital, Nigeria. Prior to the commencement of the study, ethical clearance (ADM/E 22/A/VOL VII/1047) was obtained from the Ethics Committee of the institution. The rats for the study were bred (Percie e*t al*., 2020) in the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Nigeria. This was 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). Rats weighing between 150 and 200 g were selected and grouped as an adapted sample size (Percie e*t al*., 2020; Serdar *et al*., 2021). The drugs used for the study were selected from the documented recommendations for COVID-19 infection treatment (CDCP, 2019; Calina *et al*., 2020). These drugs were procured from registered pharmacy shops, grouped and computed as follows: Group 7 (chloroquine 10mg/kg, ivermectin 200mg/kg, zinc 2.5mg/kg, azithromycin 5mg/kg, lopinavir/ritonavir 4/1mg/kg, selenium 3.33mg/kg), Group 8 (hydroxychloroquine 6.5mg/kg, ivermectin 200mg/kg, lopinavir/ritonavir 4/1mg/kg, azithromycin 5mg/kg, zinc 2.5mg/kg, selenium 3.33mg/kg), Group 9 (ivermectin 200mg/kg, lopinavir/ritonavir 4/1mg/kg, azithromycin 5mg/kg, zinc 2.5mg/kg, selenium 3.33mg/kg). The vehicle for the dissolution of the drugs was distilled water. Preparations were administered orally for 28 days using an oro-gastric tube. The animals were separated and grouped as males and females as indicators for specific cancer markers. The test groups were administered the combination drugs, and the control group was administered distilled water. After the completion of the administration, blood samples were collected after euthanization of the animals using chloroform. Toxicity markers such as glucose, lipids, liver, renal, and haematological indices were screened as described (Aghahowa *et al*., 2021).

**Determination of drug effect on cancer markers**

The Enzyme-Linked Immunosorbent Assay (ELISA) kits were used to analyze the sample for possible alteration of cancer markers. The fraction of serum samples extracted was used to assay for cancer markers according to the manufacturer's protocol described in the ELISA kits and other specified procedures (Waxman, 1995; Schrohl *et al*., 2003; Sokoll and Chan, 2004; Lindblom and Liljegren, 2000; Cooper, 2004; Wu, 2007). The cancer markers investigated have been confirmed to be in the categories of oncofetal proteins and glycoproteins (Waxman, 1995). The cancer biomarkers assessed in specific sexes were breast cancer marker (CA-15 3), ovarian cancer marker (CA-125) for female rats. Prostate cancer markers (PSA) for male rats, and liver cancer marker (AFP) for both sexes. During the procedure, all the reagents and the steps were maintained at room temperature between 20°C and 27°C. The microplate reader was

switched on before the commencement of the readings to optimize the effectiveness. The 96-well microplates were arranged for different triplicate samples as: follows: Column 1. (Standard), Column 2. (Control group), Columns 3, 4, 5 for 7, 8, 9 test groups for combination drugs. The microplate was swirled for 30 seconds to ensure mixing of the components correctly. The content was covered and allowed to incubate for 30 minutes at room temperature. The content of the microplate was then discarded by decantation. The microplate was further tapped on a dry absorbent paper. Three hundred and fifty microlitres of wash buffer was added and decanted. The procedure was repeated three times. One hundred microlitres of working substrate solution was further added to all the wells. The content was not shaken after this step. The microplate was then incubated for 15 minutes at room temperature. Fifty microlitres of stop solution was added to all the wells and mixed gently for 20 seconds. The final content of the 96-well plate was finally read in the microplate reader at absorbance wavelength of 450 nm and the reference wavelength was between 620 and 630 nm. These results were read within 30 minutes after adding the stop solution.

**Data analysis**

The data collated were analyzed using GraphPrism Version 6, San Diego, USA. They were computed as mean variants. Inferential statistics (Serdar *et al.*, 2021) were finally applied using Analysis of Variance, Tukey's and Fisher's *post hoc* tests. *P*-values equal to or less than 0.05 were noted to be significant.

## 3. RESULTS

In the study, eighteen regular toxicity markers were significantly altered. The alterations were more in group 7 combination, while groups 8, and 9 combinations had a stalemate (Fig 1 to 4). The eighteen regular toxicity markers that changed significantly were body weight (grams) (*P** Vs 8), glucose mmol/l (*P** Vs CON, 7), alkaline phosphatase U/l (*P** Vs 7,8,9), conjugated bilirubin mg/dl (*P** Vs CON, 7,8), total protein mg/dl (*P** Vs 7,9), albumin g/dl (*P** Vs 7,8,9), high-density lipoprotein mmol/l (*P** Vs 7), white blood cells $\times 10^3$µl (*P** Vs CON, 8), lymphocytes % (*P** Vs 7), granulocytes % (*P** Vs 9), lymphocytes $\times 10^3$µl (*P** Vs CON, 8), red blood cells$\times 10^6$µl (*P** Vs 7), hemoglobin g/dl (*P** Vs 7), hematocrit % (*P** Vs 7), mean cell volume fl (*P** Vs 8), red cell distribution weight (*P** Vs 9), platelets $\times 10^3$µl (*P** Vs 7, 9), platelet concentration transmittance % (*P** Vs 7, 9). Cancer Antigen -125 (*P** Vs 7, 8, 9) decreased significantly while Alpha-Fetoprotein (*P** Vs CON), Prostate Specific Antigen (*P** Vs CON, 8), and Cancer Antigen 15-3 (*P** Vs 7, 8, 9) increased significantly.

**Table 1:** Changes in Biochemical and hematological parameters

| | | Control | Group 7 | Group 8 | Group 9 | *P*-value |
|---|---|---|---|---|---|---|
| Weight (g) | | 163.8±6.34 | 139.8±10.17* | 171.8±5.50 | 145.2±4.69 | *P**Vs 8 |
| Glucose (mmol/l) | | 83.83±4.55 | 87.33±2.95 | 102.8±1.99* | 90.83±3.14 | *P**Vs CON, 7 |
| Liver indices | | | | | | |
| AST | (U/l) | 529±128.7 | 450.7±36.15 | 431.2±23.23 | 429.3±27.55 | *P* >0.05 |
| ALT | (U/l) | 446.5±147.8 | 195.7±22.13 | 208.3±26.00 | 292.2±29.92 | *P* >0.05 |
| ALP | (U/l) | 617.3±43.40* | 367.3±50.89 | 305.5±21.88 | 386.7±12.80 | *P**Vs 7, 8, 9 |
| TB | (mg/dl) | 0.47±0.07 | 0.52±0.08 | 0.53±0.08 | 0.70±0.07 | *P*>0.05 |
| CB | (mg/dl) | 0.20±0.03 | 0.20±0.04 | 0.20±0.04 | 0.35±0.03* | *P**Vs CON, 7, 8 |
| TP | (mg/dl) | 7.02±0.22* | 5.80±0.05 | 6.40±0.21 | 6.15±0.18 | *P**Vs 7, 9 |
| ALB | (g/dl) | 4.17±0.18* | 2.93±0.14 | 3.17±0.17 | 3.08±0.15 | *P**Vs 7, 8, 9 |
| Lipid indices | | | | | | |
| TC | (mmol/l) | 163.7±15.22 | 148.2±3.43 | 152.0±6.20 | 145.0±4.16 | *P*>0.05 |
| TG | (mmol/l) | 85.33±7.82 | 57.17±8.59 | 66.00±10.33 | 55.67±3.40 | *P*>0.05 |
| HDL | (mmol/l) | 47.00±2.42* | 30.67±3.55 | 40.50±4.35 | 41.67±2.60 | *P**Vs 7 |
| LDL | (mmol/l) | 94.33±15.68 | 108.7±2.46 | 98.33±6.80 | 92.17±4.64 | *P*>0.05 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Renal indices | | - | | | | |
| UREA | (mg/dl) | 41.00±4.91 | 38.33±2.14 | 40.33±4.91 | 41.67±5.05 | *P*>0.05 |
| Na | (mmol/l) | 154.7±2.16 | 155.0±3.18 | 147.3±3.05 | 152.2±2.83 | *P*>0.05 |
| K | (mmol/l) | 5.95±0.46 | 6.87±0.28 | 6.10±0.15 | 6.45±0.18 | *P*>0.05 |
| HCO3 | (mmol/l) | 21.50±0.62 | 21.50±0.36 | 21.1.53±1.54 | 20.00±0.25 | *P*>0.05 |
| Cl | (mmol/l) | 110.8±2.99 | 110.0±1.84 | 108.0±2.63 | 109.0±2.07 | *P*>0.05 |
| Creatinine | (mg/dl) | 0.62±0.17 | 0.33±0.02 | 0.33±0.02 | 0.45±0.06 | *P*>0.05 |
| | | | | | | |
| Hematological indices | | | | | | |
| WBC | $\times 10^3 \mu l$ | 3.40±1.00 | 6.57±0.36 | 4.73±0.94 | 9.35±0.96* | *P**Vs CON, 8 |
| LYM | % | 86.87±1.63* | 93.30±1.03 | 89.02±1.81 | 92.13±0.75 | *P**Vs 7 |
| MON | % | 5.70±0.36 | 4.70±0.41 | 4.17±0.54 | 4.93±0.53 | *P*>0.05 |
| GRAN | % | 6.83±1.47* | 3.43±0.48 | 6.10±1.19 | 2.63±0.32 | *P**Vs 9 |
| LYM | $\times 10^3 \mu l$ | 3.83±0.81 | 6.03±0.30 | 4.80±0.97 | 7.83±0.75* | *P**Vs CON, 8 |
| MON | $\times 10^3 \mu l$ | 0.25±0.04 | 0.30±0.04 | 0.18±0.04 | 0.40±0.11 | *P*>0.05 |
| GRAN | $\times 10^3 \mu l$ | 0.25±0.07 | 0.23±0.06 | 0.22±0.03 | 0.22±0.02 | *P*>0.05 |
| RBC | $\times 10^6 \mu l$ | 4.03±0.93* | 6.78±0.17 | 5.52±0.12 | 5.69±0.21 | *P**Vs 7 |
| HGB | g/dl | 7.75±1.94* | 13.33±0.64 | 10.37±0.44 | 11.72±0.26 | *P**Vs 7 |
| HCT | % | 22.98±3.20* | 33.53±0.99 | 28.83±0.55 | 29.72±0.93 | *P**Vs 7 |
| MCV | fl | 49.23±0.74* | 51.60±0.34 | 53.50±0.86 | 51.27±0.61 | *P**Vs 8 |
| MCH | pg | 18.68±0.76 | 20.58±0.44 | 18.60±0.65 | 19.63±0.42 | *P*>0.05 |
| MCHC | g/dl | 38.22±1.42 | 39.67±0.92 | 35.57±1.38 | 38.05±0.94 | *P*>0.05 |
| RDW-SD | - | 25.98±1.02* | 28.50±0.44 | 29.13±0.43 | 29.88±1.10 | *P**Vs 9 |
| RDW-CV | % | 14.08±0.41 | 14.80±0.24 | 15.00±0.38 | 15.52±0.48 | *P*>0.05 |
| PLT | $\times 10^3 \mu l$ | 399.0±67.81* | 976.3±156.0 | 681.3±138.7 | 1089±138.5 | *P**Vs 7, 9 |
| MPV | fl | 8.02±0.62 | 8.82±0.53 | 7.60±0.42 | 8.20±0.58 | *P*>0.05 |
| PDW | % | 8.02±0.26 | 9.02±0.42 | 8.57±0.34 | 8.78±0.33 | *P*>0.05 |
| PCT | % | 0.28±0.08* | 0.94±0.17 | 0.50±0.08 | 0.83±0.17 | *P**Vs 7, 9 |
| P-LCR | % | 4.23±4.23 | 14.23±4.50 | 3.40±3.40 | 14.93±4.74 | *P*>0.05 |

Effects of combined drugs on body weight (P* Vs 8), Glucose (P* Vs CON, 7), ALP (P* Vs 7,8,9), DP (P* Vs CON, 7,8), TP (P* Vs 7,9), ALB (P* Vs 7,8,9), HDL (P* Vs 7), WBC (P* Vs CON, 8), LYM% (P* Vs 7), GRAN% (P* Vs 9), LYM (P* Vs CON, 8), RBC (P* Vs 7), HGB (P* Vs 7), HCT (P* Vs 7), MCV (P* Vs 8), RDW-SD (P* Vs 9), PLT (P* Vs 7, 9), PCT (P* Vs 7, 9) in albino rats. Group 7: (chloroquine 10mg/kg, ivermectin 200mg/kg, zinc 2.5mg/kg, azithromycin 5mg/kg, lopinavir/ritonavir 4/1mg/kg, selenium 3.33mg/kg), Group 8: (hydroxychloroquine 6.5mg/kg, ivermectin 200mg/kg, lopinavir/ritonavir 4/1mg/kg, azithromycin 5mg/kg, zinc 2.5mg/kg, selenium 3.33mg/kg), Group 9: (ivermectin 200mg/kg, lopinavir/ritonavir 4/1mg/kg, azithromycin 5mg/kg, zinc 2.5mg/kg, selenium 3.33mg/kg). WBC: White blood cells, LY: Lymphocytes, MO: Monocytes, GR: Granulocytes, RBC: Red blood cells, HGB: Haemoglobin, PCV: Packed cell volume. MCV: Mean cell volume, MCH: Mean cell haemoglobin, MCHC: Mean cell haemoglobin concentration, RDW: Radius diameter weight, PLT: Platelets, PCT: Platelet concentration transmittance, MPV: Mean platelet volume, PDW: Platelet diameter weight, RDW: Red cell distribution weight, MPV: Mean platelet volume. P-LCR: Platelet Large Cell Ratio. RDW-SD: Red blood cell volume distribution width-SD, RDW-CV: Red blood cell volume distribution width-CV, HCT: Hematocrit

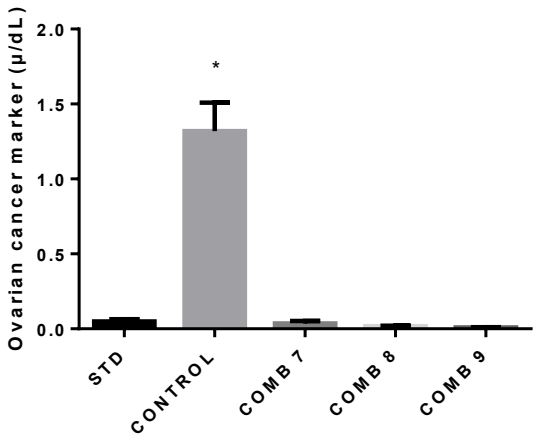


**Fig 1. Effect of combination Drugs recommended for COVID-19 on Ovarian cancer marker (Ca-125) (P* Vs STD, 7,8,9)**

**Group 7: (chloroquine 10mg/kg, ivermectin 200mg/kg, zinc 2.5mg/kg, azithromycin 5mg/kg, lopinavir/ritonavir 4/1mg/kg, selenium 3.33mg/kg), Group 8: (hydroxychloroquine 6.5mg/kg, ivermectin 200mg/kg, lopinavir/ritonavir 4/1mg/kg, azithromycin 5mg/kg, zinc 2.5mg/kg, selenium 3.33mg/kg), Group 9: (ivermectin 200mg/kg, lopinavir/ritonavir 4/1mg/kg, azithromycin 5mg/kg, zinc 2.5mg/kg, selenium 3.33mg/kg).**

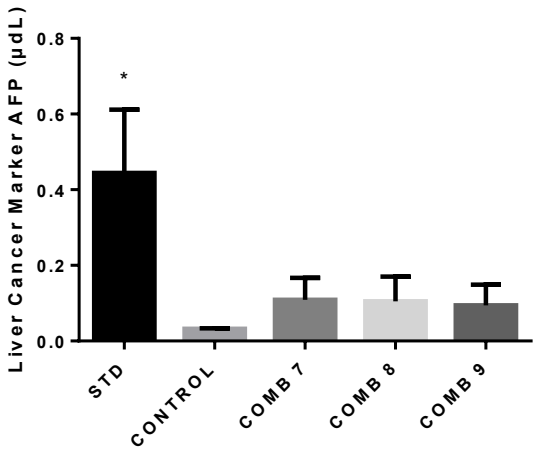


**Fig 2: Effect of Combination Drugs recommended for COVID-19 on Liver Cancer Marker (AFP) (P* Vs CON)**

**Group 7: (chloroquine 10mg/kg, ivermectin 200mg/kg, zinc 2.5mg/kg, azithromycin 5mg/kg, lopinavir/ritonavir 4/1mg/kg, selenium 3.33mg/kg), Group 8: (hydroxychloroquine 6.5mg/kg, ivermectin 200mg/kg, lopinavir/ritonavir 4/1mg/kg, azithromycin 5mg/kg, zinc 2.5mg/kg, selenium 3.33mg/kg), Group 9: (ivermectin 200mg/kg, lopinavir/ritonavir 4/1mg/kg, azithromycin 5mg/kg, zinc 2.5mg/kg, selenium 3.33mg/kg).**

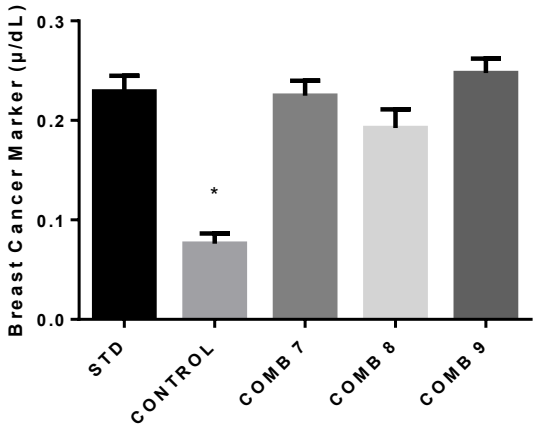


**Fig 3: Effect of Combination Drugs recommended for COVID-19 on Breast Cancer Markers (Ca 15-3) (*P** Vs STD 7,8,9)**
**Group 7: (chloroquine 10mg/kg, ivermectin 200mg/kg, zinc 2.5mg/kg, azithromycin 5mg/kg, lopinavir/ritonavir 4/1mg/kg, selenium 3.33mg/kg), Group 8: (hydroxychloroquine 6.5mg/kg, ivermectin 200mg/kg, lopinavir/ritonavir 4/1mg/kg, azithromycin 5mg/kg, zinc 2.5mg/kg, selenium 3.33mg/kg), Group 9: (ivermectin 200mg/kg, lopinavir/ritonavir 4/1mg/kg, azithromycin 5mg/kg, zinc 2.5mg/kg, selenium 3.33mg/kg).**

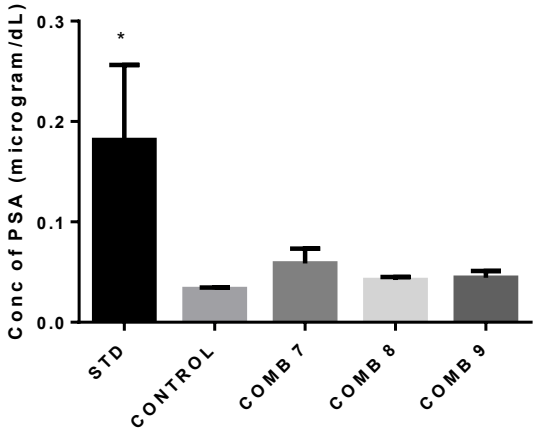


**Fig 4: Effect of combination Drugs recommended for COVID-19 on Prostate Specific Antigen (PSA) (P* Vs CON,8)**
**Group 7: (chloroquine 10mg/kg, ivermectin 200mg/kg, zinc 2.5mg/kg, azithromycin 5mg/kg, lopinavir/ritonavir 4/1mg/kg, selenium 3.33mg/kg), Group 8: (hydroxychloroquine 6.5mg/kg, ivermectin 200mg/kg, lopinavir/ritonavir 4/1mg/kg, azithromycin 5mg/kg, zinc 2.5mg/kg, selenium 3.33mg/kg), Group 9: (ivermectin 200mg/kg, lopinavir/ritonavir 4/1mg/kg, azithromycin 5mg/kg, zinc 2.5mg/kg, selenium 3.33mg/kg).**

## 4. DISCUSSION

The alteration in weight as seen in Table 1, may be due to the nutritional components of the palletized feeds taken during the study. There could be variation in feeding habit in all the groups irrespective of the drug combination administered. These changes could also be due to the inherent features of individual drugs in the combination. Since there was a since there was a significant increase in weight, group 7 drug combination could be of benefit to co-morbid patients that are obessed, while group 8 combination could be of risk to obese patients, while beneficial to individuals that have lean body mass. There were more changes in toxicological parameters in

group 7 in comparisons with groups 8 and 9. In other words, drug combinations in group 7 caused more changes in parameters, while drug combinations in groups 8 and 9 caused less changes in parameters. These findings underscore the potential interplay between drug treatments, metabolic dysregulation, and cancer risk, highlighting the importance of considering weight changes in therapeutic assessments. The peculiarities of some of the drugs in combinations unraveled the benefits in the combination. Therefore, the results suggest full adoption in areas where there may be potential benefits.

The results of the lipids except HDL supports the safety of the combination drugs in lipid disorders. However, elevated levels of cholesterol in the blood have been linked to a higher likelihood of cancer development through several pathways. In addition, different byproducts of cholesterol metabolism have shown a positive association with the proliferation and spread of cancer cells (Patel and Kashfi, 2021). Therefore, there is need for caution in individuals that may have been diagnosed of cancer. Since there were no significant changes in renal parameters, the combinations may not worsen renal failure or cause ionic derangement. This phenomenon may not be the same in subjects that are dose sensitive and in chronic studies. Therefore, caution is needed in the use of the combinations in dose sensitive individuals. Elevation of red blood cells parameters in group 7 suggest a beneficial effects for patients that may be anemic in co-morbidities. Elevation of white blood cells parameters may be due to oxidative stress induced by the drug combinations doses and viral organisms. These significant changes may also be due to the contributory effects of individual drugs in the combinations. Elevated blood glucose levels, or hyperglycemia, may indirectly impact cancer cells by triggering increased levels of insulin/IGF-1 and inflammatory cytokines in the bloodstream that may enhance cell proliferation. In addition, there are evidences suggesting that hyperglycemia could directly influence cancer cell proliferation, apoptosis, and metastasis (Vigneri *et al*., 2009; Johnson *et al*., 2012; Suh and Kim, 2011). Therefore, elevation seen in this study may be a risk factor in worsening COVID-19 co-moridities with cancer. In addition, high glucose levels have been documented to activate multiple signaling pathways that could influence various aspects of cancer cell behavior, including proliferation, migration, invasion, and recurrence (Duan *et al*., 2014). Moreover, epigenetic modifications of oncogenic pathways induced by high glucose could lead to sustained activation of cancer cell proliferation (Siebel *et al*., 2010; Cencioni *et al*., 2014). However, these direct effects of high glucose on cancer cell behavior remain relatively underexplored, necessitating further investigation into the involved signaling pathways and their regulation (Ryu *et al*., 2014).

COVID-19 and diabetes as co-morbidities have been reported (Wang *et al.,* 2020c). Therefore, group-8 combination may be more risky as seen in Table 1. In diabetic patients, infection, such as with COVID-19, can elevate stress hormone levels like glucocorticoids and catecholamines, resulting in high glucose levels (hyperglycaemia). It is also interesting to note that higher glucose levels have been shown to both directly stimulate SARS-CoV-2 replication in human monocytes, maintaining SARS-CoV-2 replication by producing reactive oxygen species and activate hypoxia-inducible factor-1$\alpha$ (Codo *et al.,* 2020). The study therefore suggests caution in the use of combination due to possible elevation of serum glucose in all the combinations as seen in Table 1. Effect of combination drugs recommended for COVID-19 on Ovarian cancer marker (Ca-125) (*P** Vs STD, 7, 8, 9). There was a significant reduction in the 7, 8, 9 combination as in Fig. 1. The marked reduction in the concentration of Ca-125 marker may be beneficial to individuals that had existing ovarian cancer. This could be due to individual drugs or the combination. There was a significant elevation in the concentration of Liver Cancer Markers due to the combinations in groups 7, 8, 9. This therefore mean that these combinations could worsen co-morbid condition in subjects that may have existing liver cancer. Elevations seen in groups 7, 8, 9 could pose a risk in patients with existing liver cancer when on any of the combinations. Or the combinations could mask therapy for individuals who are already responding to treatment. On the contrary, there could also be hepatocellular benefit as seen in the reduction of alkaline phosphatase, conjugated bilirubin, total protein, and albumin parameters as in Table 1. Therefore, any combination of these suggests hepato-protective mechanisms for subjects at risk of hepatocellular damage. AFP levels are typically low in healthy adults; heightened concentrations of AFP in the bloodstream have been frequently linked to hepatic cancer (Forner *et al*., 2018). In the study, all the drugs used were able to raise the AFP levels compared to the control group. The findings of this study showed that there was a significant difference in the mean concentration of the liver cancer marker (AFP) after the administration of the various COVID-19 test drugs. This was in line with a study conducted that showed that chloroquine treatment could induce mitochondrial apoptosis in liver cancer cells, and a single treatment of chloroquine could suppress the growth of liver cancer cells, leading to low levels of AFP (Hu *et al*., 2016). It could also be observed that the hydroxychloroquine combination group had a similar alteration as in Fig. 2, which could be similar to previous findings in tumor suppression activity through autophagy (Mao *et al*., 2018). Studies on ivermectin have shown its potential anti-cancer effect on major types of liver cancer (Lu *et al*., 2022), and this would have led to the reduction of AFP as seen in all the groups. Lopinavir/Ritonavir may have also influenced the alteration. Elevated levels of these enzymes in previous studies indicate liver damage, which can be associated with liver cancer (Zhang *et al*., 2020). Russo *et al*., (2004), described a case of severe hepato-toxicity in a

patient receiving azithromycin for respiratory tract infection, in which there was an increase in the concentration of ALT. This study suggests that the combination with azithromycin may have a contrary advantage. There was a significant elevation in the concentration of Breast Cancer Markers (Ca-15 3) due to the groups 7, 8, 9 combinations. It therefore mean that these combinations could worsen co-morbid condition in subjects that may have been diagnosed of breast cancer. The effect may be less due to the group 8 combination. Breast cancer individuals seem to be more at risk in the use of the combined drugs as shown in this study. Elevations due to the combinations may be due to the inherent effect of individual drugs in the combination. This however that a patient that may have breast cancer should avoid any form of the combination therapies. Since there was a significant elevation in the concentration of PSA due to groups 7, 8, 9 combinations when compared with the control group, these combinations could worsen co-morbid condition of the subjects that may be diagnosed of prostate cancer. The effect may be less due to the group 8 combination. The elevation seen may not necessarily be due to the existence of cancer (Malati, 2007; NCI, 2023) but may be due to individual drugs or the combined drugs. The elevation that was less in PSA cancer marker level compared with breast cancer may be due to feminine hormonal alteration and social factors that are associated with cancer. The prostate gland produces a protein known as prostate-specific antigen (PSA) that is commonly screened as a biomarker in prostate cancer diagnosis (Catalona *et al*., 1991).

George *et al.* (2017) suggested that hydroxychloroquine may have some activities on PSA levels. Chloroquine had moderate levels of PSA, which goes with studies that have discussed the potential use of chloroquine in the treatment of prostate cancer by autophagy inhibition (Gabriele *et al*., 2018). The combination drugs in group 9 could have minimal impact on the prostate gland that is relevant in cases of already existing prostate cancer disease. Lower levels due to the group 9 combination may suggest that the drugs do not exacerbate prostate inflammation. Group 8 combination has a greater impact on the prostate gland and can exacerbate prostate cancer when used in COVID-19 therapy. The changes may be due to individual variations in the rats and also the length of time of administration of the combination drugs. It is important to note that elevated levels of PSA may not be indicative of prostate cancer. The exact cause of the correlation between increased weight and cancer risk remains unclear. Still there is increasing evidence that increased weight could be influenced significantly by lipids'. Therefore, the effect on lipid metabolism may play a role in the development of tumors (Leroith *et al*., 2008). Individual drugs may have inherent influence that cannot be underrated. Chloroquine has been documented to disrupt some processes leading to cancer cell death (Liang *et al*., 2016). Furthermore, studies have explored chloroquine efficacy in combination with other anti-cancer drugs. In ovarian cancer cell lines, chloroquine counteracts CDDP-induced cell proliferation by inhibiting autophagy activation through the Akt/mTOR signaling pathway (Zhu *et al*., 2017). Additionally, combining CQ with an mTOR inhibitor could enhance the radiosensitivity of colon cancer (Shiratori *et al*., 2019). Ivermectin had been previously reported induced the ubiquitination-mediated degradation of the oncogenic kinase PAK1 in ovarian and glioblastoma cancer cell lines (Liu *et al*., 2016; Crump, 2017). Therefore, the combination of ivermectin with other recommended drugs may have an added advantage. PAK1 plays a pivotal role in cytoskeletal reorganization and nuclear signaling, contributing to tumor growth in over 70% of human cancers (Liu *et al*., 2016). The reduction of PAK1 levels could lead to the inhibition of the Akt/mTOR pathway, a suppressor of autophagy, as proven by decreased phosphorylation levels of Akt, mTOR, p70S6K, and 4EBP1, that is mediated through direct interaction between PAK1 and Akt. Furthermore, ivermectin had enhanced the interaction with Beclin 1 as positive regulators of autophagy, such as Atg14L and Vps34, while reducing its interaction as negative regulators like Bcl-2 (Dou *et al*., 2016). In the research conducted in 1996, it was found that ivermectin treatment in murine multidrug-resistant (MDR)-P388 and human MDR-CEM leukemia cells, the drug acted as both a substrate and inhibitor of P-glycoprotein-mediated multidrug resistance in cancer (Didier and Loor, 1996). Additionally, another study revealed that doses of ivermectin ranging from 3 to 5 mg/kg effectively could inhibit the growth of human melanoma and various other cancer xenografts in mice (Driniaev *et al*., 2004). Collectively, these findings demonstrated that there may be tumor preventing effects of ivermectin, which aligns with the possible observed low cancer induction potential in this present study as seen in the combination also in the documented activity against SARS-CoV-2 (Yang *et al.*, 2019, Matsuyama *et al.*, 2020, Mody *et al.*, 2021). Combination with the recommended agent may act synergistically in enhancing the observed therapeutic advantage rather than mono-therapies. There may also be potentiation of adverse effects. Studies have explored chloroquine efficacy in combination with other anti-cancer drugs, showing promising results in breast and colon cancers (Zhu et al., 2017; Shiratori et al., 2019). Despite the cytotoxic effects observed with combined CQ and anti-cancer drugs, the precise mechanisms remain elusive, potentially explaining the relatively low induction of breast cancer markers observed in the study. Previous research indicates a favorable safety profile of azithromycin in cancer patients, despite the limited availability of preventive measures against SARS-CoV-2 infections (Mair et al., 2023). Azithromycin has shown anti-proliferative effects in various cancer cell lines, particularly when combined (Qiao et al., 2018). These findings align with the study's indication of the limited potential for inducing breast cancer markers.

Ivermectin has been shown to induce the degradation of the oncogenic kinase PAK1 in breast and glioblastoma cancer cell lines (Liu et al., 2016). In addition, ivermectin has demonstrated inhibitory effects on multidrug-resistant leukemia cells and the growth of various cancer xenografts in mice without adverse effects (Didier and Loor, 1996; Driniaev et al., 2004). Therefore, the combination with other drugs could be beneficial. According to Sasaki *et al* (2010), the use of chloroquine in combination with other chemotherapeutic reagents may ameliorate the induction of some cancers despite reported statistical fatalities (WHO, 2022). In some selected cases based on prevalence (WHO, 2014), many factors may encompass genetics, epigenetics, and environmental exposures (Watson *et al*., 2019). These biomarkers may be expressed in several discernible stages, namely initiation, promotion, and progression, culminating in the emergence malignant tumours (Hanahan & Weinberg, 2011), leading to metastasis (Lambert *et al*., 2017; American Cancer Society, 2022; Pritchard *et al*., 2017; De Laere *et al*., 2017; Loeb *et al*., 2014; De Marzo *et al*., 2007). In summary, research on the potential induction of markers of cancer by drugs recommended for COVID-19 treatment would have significant implications in clinical practice and public health, considering the efficacy and safety of the drugs. Healthcare professionals will therefore need evidence-based information to make informed decisions in the treatment strategies. Findings in this study can inform public health policies makers on the guide in the safety, and the appropriate use of COVID-19 treatments on a global scale (WHO, 2021). Also findings have unraveled that these combination drugs further validated the adoption in the treatment guideline for the treatment of COVID-19 infection.

## 5. CONCLUSION

The findings in this study revealed some significant effects of combination drugs recommended for the treatment of COVID-19 infection on some common toxicity and cancer biomarkers. Since these annotated results had shown changes, caution needs to be taken in the use of the combination drugs in the treatment of COVID-19 infection. The data therefore serves as a template for future review of the treatment policy regulating COVID-19 infection.

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**Authors' Contributions**

Sylvester Aghahowa, Michael Aghahowa, Frank Amegor, Humphrey Osadolor: - Designed the study, supervised every stage of the study and the writing of the manuscript. Anthonia Ebosele, Michael Asogun, Paul Ehikhuemen, Josephine Uwadione, Joy Ikhimioya, Michael Ogabo, Charles Oluwabusuyi, Blessing Unukpo, Daniel Ese, Sydney Onwudiwe, Franklyn Agu, Bello Onome, Jessica Andy-Omezi, Favor Eshiobugie, Justine Ojonugwa, Osahon Enadeghe. Philip Obarisiagbon, Osagie Eremwanarue, Paul Aikorogie: Assisted at every stage in the methodology.

**Ethical Approval**

In this article, the animal regulations are followed as per the ethical committee guidelines of Department of Pharmacology and Toxicology, University of Benin, Nigeria and the Department of Chemical Pathology, University of Benin Teaching Hospital, Nigeria; the authors observed the Cancer Markers Induction Potential and Adverse Effects of Anti-COVID-19 Drugs in *Rattus norvegicus*. Ethical clearance (ADM/E 22/A/VOL VII/1047) was obtained from the Ethics Committee of the institution. The Animal ethical guidelines are followed in the study for observation, identification & experimentation.

**Informed Consent**

Not applicable.

**Conflicts of interests**

The authors declare that they have no conflicts of interests, competing financial interests or personal relationships that could have influenced the work reported in this paper.

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**Data and materials availability**

All data associated with this study will be available based on the reasonable request to corresponding author.

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