Original Article

Evaluation of the Effects of Co-Administration of Ivermectin and Hydroxychloroquine on the Blood Parameters of Wistar Rats

***[*]Eseine-Aloja CE[1], Izunya AM[2], Ogaegbe PI[2], Ediale EO[2], Oni SI[2], Osawaru E[2], Ebhojaye KI[3], Ujaddughe ME[4], Eseine CO[5], Ujaddughe OM[2,6].**

[1] *Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Ambrose Alli University, Ekpoma, Nigeria.*

[2] *Department of Anatomy, Faculty of Basic Medical Sciences, Ambrose Alli University, Ekpoma Nigeria.*

[3] *Anatomy Department, Faculty of Basic Medical Sciences, University of Port Harcourt, Rivers State, Nigeria.* [4]*Gateshead Health NHS Foundation Trust, England, United Kingdom.*

[5] *Department of Nursing, Faculty of Basic Medical Sciences, Ambrose Alli University, Ekpoma Nigeria.* [6]*School of Anatomical Sciences, University of the Witwatersrand, Johannesburg, South Africa.*

Article History

Submitted: 24/05/2024; Accepted: 11/06/2024: Published: 23/06/2024

***Correspondence:** Eseine-Aloja CE.

Email: *cee-aloja@aauekpoma.edu.ng*

ABSTRACT

In response to the emergence of SARS-CoV-2, in-vitro studies and clinical observations have provided evidence to support the use of Hydroxychloroquine (HCQ) and ivermectin in the treatment and prevention of COVID-19 leading to a surge in their therapeutic application. Blood is widely used in toxicological research as an indicator of physiological and pathological changes. The aim of this study was to determine the combined effect of co-administration of ivermectin and HCQ on blood parameters. In this study, an experimental design was adopted using eighty-four wistar rats randomly divided into seven groups (Groups A to G). Rats were administered HCQ, Ivermectin and a combination of both therapeutic agents; the rats were sacrificed after drug administration for a period of five, fourteen and twenty-eight days for analysis of blood parameters. Findings of this study revealed that HCQ and ivermectin interfered with hemoglobin concentration, WBC count, RBC count and PCV when compared to control. On the other hand, platelet counts were significantly higher at low doses of the therapeutic agents and significantly reduced when HCQ and ivermectin were co-administered at high doses. Furthermore, hematological indices showed elevated levels of MCV in group D (61.73 ± 0.21fl), E (59.77 ± 0.06fl) and G (60.63 ± 0.12fl) while significantly higher MCH values were observed in groups D (21.83 ± 0.35pg) and E (22.30 ± 0.30pg). Most of these findings are indicative of iron deficiency anemia, vitamin B deficiency induced anemia and hemolytic anemia possibly in response to drug toxicity.

Keywords: Blood parameters, Co-administration, Hydroxychloroquine, Ivermectin.

INTRODUCTION

Blood is a constantly circulating fluid through blood vessels (arteries and veins) that provides the body with nutrition, oxygen, and waste removal.[1] Blood is widely used in toxicological research as a promising indicator of physiological and pathological changes. Hematological parameters namely; hemoglobin (Hb), Hematocrit (Hct), Red Blood Cells (RBCs), White Blood Cells (WBCs) and platelets as well as hematological indices namely; mean cellular volume (MCV) and mean cellular hemoglobin concentration (MCHC) can serve as health indicators.[2] Diagnosis with data obtained from blood examination is made possible because hematological parameters and indices are often tightly regulated and under constant genetic control, therefore, alterations of these parameters indicate some forms of physiological and pathological changes.[3]

HCQ, an analog of chloroquine (CQ) is an

Article Access

Website: www.wjmbs.org

10.5281/zenodo.12802881

How to cite this article

**Eseine-Aloja CE, Izunya AM, Ogaegbe PI, Ediale EO, Oni SI, Osawaru E, Ebhojaye KI, Ujaddughe ME, Eseine CO, Ujaddughe OM.. Evaluation of the Effects of Co-Administration of Ivermectin and Hydroxychloroquine on the Blood Parameters of Wistar Rats. West J Med & Biomed Sci. 2024;5(2):93-103. DOI:10.5281/zenodo.12802881*

immunomodulator used for the treatment of malaria. It acts by interference with the digestion of haemoglobin in the erythrocytic stages of the malaria life cycle.[4] In recent times, HCQ has been used in the treatment of rheumatoid arthritis and systemic lupus erythematosus. In the treatment of rheumatoid arthritis, HCQ increases the pH within intracellular vacuoles and alters processes such as protein degradation by acidic hydrolases in the lysosome, assembly of macromolecules in the endosomes, and post-translation modification of proteins in the Golgi apparatus.[5] Over the years, Ivermectin has remained the mainstay for the elimination of onchocerciasis and lymphatic filariasis; two global disfiguring and devastating diseases that blight the lives of billions of the poor and disadvantaged populations throughout the tropics. Ivermectin has equally been used to treat a variety of internal nematode infections, including onchocerciasis, strongyloidiasis, ascariasis, cutaneous larva migrans, filariases, gnathostomiasis and trichuriasis, as well as in the oral treatment of ectoparasitic infections, such as pediculosis and scabies.[6]

In 2019 severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) also known as coronavirus disease 19 (COVID-19) was first detected in Wuhan, China[7] and immediately spread across all countries of the world, hence its timely classification by the WHO as an epidemic.[8,9]The high infection rate and death toll associated with COVID-19 coupled with the absence of any known treatment heightened the quest for an urgent therapeutic strategy to control the spread of the novel COVID-19 virus and treat ailing patients infected by it,[10] hence many studies on treatments for COVID-19 were championed by researchers. From the research conducted as in-vitro studies and clinical observations described by some as "basic and inconsistent",[11] evidence to support the use of HCQ and ivermectin in the treatment and prevention of COVID-19 emanated. One of such research conducted as an in-vitro study showed that ivermectin produces a 99% reduction in the COVID-19 viral load within 48–72 hours.[11]In another study, the antiviral action of ivermectin against the SARS-CoV-2 clinical isolate in-vitro was demonstrated, with a single dose of the drug able to control viral replication within 24 to 48 hours possibly as a result of the inhibition of importin-α/β1–mediated nuclear import of viral proteins, as shown for other RNA viruses.[12] As Ivermectin has been stated to act as a specific inhibitor of importin-α/β–mediated nuclear import.[13] Thus, by impacting on importin-α/β–dependent nuclear transport of viral proteins, ivermectin suppresses the replication of several RNA viruses.[12] Similarly, in-vitro studies of the effect of HCQ on COVID-19 have also been reported to show antiviral effects at both pre-infection and post-infection stages of the corona virus.[14] Its mode of action is thought to be by interference with the glycosylation of angiotensin-converting enzyme 2, thereby reducing the binding efficiency between angiotensin-converting enzyme 2 on host cells and the SARS-CoV-2 spike protein.[15] These findings therefore encouraged the administration of ivermectin and HCQ either separately or together in the prevention and treatment of COVID- 19.

While the administration of these drugs is geared at saving lives is remarkable, it is worthy of note that the administration of HCQ or ivermectin as well as the co-administration of both agents in the prevention and treatment of COVID-19 is without the approval of Federal Drug Administration, National Institutes of Health, World Health Organization, Public Health Officials and majority of Health Care providers.[16] This is particularly because, there is no concrete proof of its effectiveness in COVID-19 treatment or clear definition of the possible effects of the co-administration of both drugs (ivermectin and HCQ) on the health of an individual during and after treatment.

However, in spite of the limited evidence of the effectiveness of HCQ and ivermectin in the prevention and treatment of COVID-19, a rise in prescriptions and sales of both drugs during the COVID-19 scare was observed by researchers in several countries across the globe.[17,18,11] This increased demand for both drugs was also experienced in Nigeria and is evident in the price hike of both drugs during same period. In Nigeria,

the Presidential Task Force (PTF) on COVID-19 pandemic raised the alarm over the attitude of some Nigerians who were buying hydroxychloroquine and ivermectin in large quantities and even storing the drug.[19]These findings indicate an increased intake of ivermectin and hydroxychloroquine in the Nigerian population as well as other populations of the world and the possibility of a co-administration of both drugs either on the instruction of physicians or as a result of self-medication. Hence the necessity to evaluate the effect of the administration and co-administration of ivermectin and HCQ on blood parameters; specifically on the hematological parameters namely, hemoglobin concentration, red blood cell count, white blood cell count, neutrophils, lymphocytes, monocytes, packed cell volume and platelets as well as on the hematological indices namely mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, using Wistar rats as a way of identifying and predicting the effects of the administration and co-administration of both drugs on humans.

MATERIALS AND METHODS

This study adopted an experimental research design using wistar rats. Eighty-four (84) adult Wistar rats of average weight of 150g upon purchase from Animal Holdings of the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin city, Nigeria were randomly assigned into seven groups; groups A to G making a total of 12 rats per group (n = 12). Group A served as control while groups B to G served as treatment groups. Acclimatization lasted a period of 15 days. Materials/agents used in this study include Rat feed (CHIKUN FEED), Rat cage, Hydroxychloroquine (HCQ), Ivermectin, Hand gloves, Gavage, Sample collection tubes, Syringe and Needle, Automated Hematology Analyzer.

Drug administration was done by direct feeding using a gavage for a period of 28 days as specified in Table 1. The day of treatment commencement (day 16 of rat arrival) was therefore taken as treatment day 1 (Td_1)

Table 1: Dosage of HCQ and Ivermectin

Group	Agent	Dosage	
		Day 1 and 2	Day 3 to 28
A (Control)	-------	-------	-------
B	HCQ	6.66mg/kg body weight **bd**	3.33mg/kg body weight **bd**
C	HCQ	13.33mg/kg body weight **bd**	6.66mg/kg body weight **bd**
D	Ivermectin	0.2mg/kg body weight **od**	
E	Ivermectin	0.4mg/kg body weight **od**	
F	HCQ	6.66mg/kg body weight **bd**	3.33mg/kg body weight **bd**
	+ Ivermectin	0.2mg/kg body weight **od**	
G	HCQ	13.33mg/kg body weight **bd**	6.66mg/kg body weight **bd**
	+ Ivermectin	0.4mg/kg body weight **od**	

Blood sample for analysis were collected from three rats/group on TD_5, TD_{14} and Td_{28} of the experiment. [20]Blood sample collected was analysed to determine the hematological parameters and indices using an automated hematology analyzer.[21]

Obtained data were analyzed using Statistical Product and Service Solutions (SPSS) version 21. One-way analysis of variance (ANOVA) to determine the difference in the mean between the groups. Tukey's Honest Significant Difference (HSD) test was used to determine the statistical significance of observed differences at 0.05 level of significance. Results were expressed as Mean ± Standard Error of Mean (SEM).

Ethical approval for this study was obtained from Ambrose Alli University Research Ethics Committee, (REC).

RESULTS

Findings from this study on the hematological parameters and indices of wistar rats are presented below in a logical order in Table 2. Note that * implies that the calculated mean is statistically significantly different from control at the 0.05 level.

Singular and co-administration of HCQ and Ivermectin caused a statistically significant decline in hemoglobin concentration on TD_5 in Groups C (8.77 ± 0.21g/dL), D (10.17 ± 0.29g/dL), E (8.53 ± 0.06g/dL) and G (6.80 ± 0.10g/dL). Similarly, a statistically significant decline in RBC counts as observed in group C ($4.24 \pm 0.03 \times 10^6/\mu L$), D ($4.66 \pm 0.07 \times 10^6/\mu L$), E ($3.82 \pm 0.03 \times 10^6/\mu L$) and G ($3.39 \pm 0.06 \times 10^6/\mu L$) on TD_5 while in group F a statistically significant increase in RBC count of $6.37 \pm 0.26 \times 10^6/\mu L$ was observed.

WBC count of the control group was observed to

increase as the days progressed form 5833.33 ± 1289.70/mm^3 on TD_5 to 8833.33 ± 251.66/mm^3 on TD_{14} and 14600.33 ± 100.00/mm^3 on TD_{28}. In Group B, WBC count of 11900 ± 556.88/mm^3 was recorded on TD_5, followed by a decline to 9000.00 ± 1000.00/mm^3 on$TD_{14}$and apeak of 15300.00 ± 200.00/mm^3 on TD_{28}. The WBC counts on$TD_{14}$and $TD_{28}$of group B did not differ significantly from control. In group C there was a continuous increase in WBC count as the days progresses from 4533.33 ± 57.74/mm^3, 11600 ± 300.00/mm^3 and 13200.00 ± 200.00/mm^3 on TD_5, TD_{14} and TD_{28} respectively as observed in the control group. In groups C, D, E and G there was a decline in WBCs on TD_5 followed by a rapid increase on TD_{14} and then a decrease on TD28 (except in group E where the increase continued).

Neutrophil count in the Control group increased throughout the period of the experiment from 7.67 ± 0.58% on TD_5 to 10.00 ± 2.00% on TD_{14} and eventually its peak of 10.33 ± 3.06% on TD_{28}. When compared with the control group, a statistically significant decline in lymphocyte count was observed in group G (5.33 ± 0.58%) on TD_5 and groups E (5.67 ± 1.53%), F (4.67 ± 2.31%) and G (6.33 ± 0.58%) on TD_{14} while a statistically significant increase in lymphocyte count from control was observed in group D (12.67 ± 0.58%) on TD_5.

Lymphocyte count differed statistically from control in group D on TD_5 (70.33 ± 0.58%) where a decline was recorded but in groups E (88.33 ± 2.08%) and F (87.67 ± 1.53%) on TD_{14} an increase was recorded.

Monocyte count was found to increase from 8.67 ± 0.58% on TD_5 to 13.00 ± 1.00% on TD_{14} followed by a decline to 11.00 ± 3.46 on TD_{28} in the control group. The monocyte count observed in this study on TD_5 when compared with the control, revealed that the monocyte count in all treatment groups were higher. This increase in monocyte count was statistically significant in groups B (10.67 ± 0.58%), C (12.67 ± 1.15%) and D (17.33 ± 0.58%) with the increase most notable in group D. The monocyte count was statistically significantly less than control in groups D (9.00 ± 2.00%), E (6.67 ± 0.58%) and F (8.33 ± 0.58%) on $TD_{14}$and in group G (7.00 ± 0.00%).

The PCV of rats in the control group were within the range of 31.33 ± 3.06% and 34.33 ± 1.53%. On TD_5, demonstrating a statistically significant decrease in PCV when compared to what was observed in groups C (24.00 ± 0.00%), D (28.33 ± 0.58%), E (22.33 ± 0.58%) and G (20.33 ± 0.58%). On TD_{14}, a statistically significant increase in PCV was observed in group D (38.67 ± 0.58%).

This study showed that there was an increase in platelet count as the treatment days progressed from 1.35 ± 0.21 x 10^5/µL on TD_5 to 1.88 ± 0.04 x 10^5/µL on TD_{14} and 3.83 ± 0.68 x 10^5/µL on TD_{28}. On TD_5 when compared with control. Groups B, D and F had statistically significant increase in platelet counts of 2.93 ± 0.29 x 10^5/µL, 2.90 ± 0.07 x 10^5/µL and 3.06 ± 0.23 x 10^5/µL respectively while in group G platelet count was significantly reduced (0.42 ± 0.07 x 10^5/µL). On TD_{14} groups B, D and G had statistically significantly increased reduced platelet counts of 7.07 ± 0.60 x 10^5/µL, 4.93 ± 0.63 x 10^5/µL and 5.48 ± 0.46 x 10^5/µL. On TD_{28}, the platelet count in all treatment groups was statistically significantly higher that control.

MCV of wistar rats recorded in the control group of this study is found to be within the range of 53.67 ± 0.81fl and 56.70 ± 1.14fl. Statistically significant elevated levels of MCV were observed in groups D (61.73 ± 0.21fl), E (59.77 ± 0.06fl) and G (60.63 ± 0.12fl) on TD_5 and group B (60.27 ± 2.60fl) on TD_{28}. MCH values of control in this study ranged from 19.20 ± 0.20pg to 21.53 ± 0.21pg. In comparison with control on TD_5 groups D (21.83 ± 0.35pg) and E (22.30 ± 0.30pg) as well as group F (21.80 ± 2.33pg) on TD_{28} had significantly higher MCH values. On TD_{14}, a similar comparison with control revealed that groups B (19.27 ± 2.35pg) and E (18.43 ± 0.55pg) had significantly less MCH than control. MCHC of wistar rats have been reported in this study to range from 35.40 ± 0.36g/dL to 39.90 ± 0.35g/Dl. Lower MCHC than control was observed in group G (33.10 ± 0.66g/dL) on TD_5, groups B to G on TD_{14} and group B (33.17 ± 0.57g/dL) on TD_{28}. On the other hand, on TD28, group G had significantly higher MCHC values of 38.70 ± 0.46g/dL.

Table 2: Effect of HCQ and Ivermectin on hematological paramenters and indices

1. Effect of HCQ and ivermectin administration on Hemoglobin

TREATMENT GROUP	Hemoglobin		
	TD_5	TD_{14}	TD_{28}
A (Control)	12.43 ± 0.31	12.30 ± 1.10	12.77 ± 0.06
B	12.13 ± 0.15	12.03 ± 0.40	13.07 ± 0.12
C	8.77 ± 0.21*	12.67 ± 1.53	13.03 ± 0.35
D	10.17 ± 0.29*	14.70 ± 0.52	11.80 ± 0.87
E	8.53 ± 0.06*	10.43 ± 1.85	12.67 ± 1.31
F	12.40 ± 0.36	11.67 ± 1.75	13.60 ± 1.00
G	6.80 ± 0.10*	10.57 ± 2.21	12.73 ± 0.70

2. Effect of HCQ and ivermectin administration on RBC count

TREATMENT GROUP	RBC COUNT (x 10^6/µL)		
	TD_5	TD_{14}	TD_{28}
A (Control)	5.98 ± 0.16	5.73 ± 0.49	5.92 ± 0.84
B	6.01 ± 0.06	6.45 ± 0.99	6.31 ± 0.73
C	4.24 ± 0.03*	5.85 ± 0.59	6.74 ± 0.16
D	4.66 ± 0.07*	6.90 ± 0.15	6.02 ± 0.45
E	3.82 ± 0.03*	5.50 ± 0.94	6.28 ± 0.37
F	6.37 ± 0.26*	6.40 ± 0.79	6.80 ± 0.40
G	3.39 ± 0.06*	5.43 ± 1.11	6.25 ± 0.33

3. Effect of HCQ and ivermectin administration on WBC count

TREATMENT GROUP	WBC COUNT (/mm^3)		
	TD_5	TD_{14}	TD_{28}
A (Control)	5833.33 ± 1289.70	8833.33 ± 251.66	14600.00 ± 100.00
B	11900.00 ± 556.78*	9000.00 ± 1000.00	15300.00 ± 200.00
C	4533.33 ± 57.74	11600.00 ± 300.00*	13200.00 ± 173.21
D	3933.33 ± 152.75*	13500.00 ± 351.19*	8000.00 ± 721.11*
E	4700.00 ± 173.21	7400.00 ± 916.52*	10900.00 ± 763.76*
F	10366.67 ± 723.42*	7200.00 ± 360.56*	12700.00 ± 1850.23
G	2400.00 ± 173.21*	13900.00 ± 173.21*	10800.00 ± 300.00*

4. Effect of HCQ and ivermectin administration on Neutrophil count

TREATMENT GROUP	NEUTROPHIL COUNT (%)		
	TD_5	TD_{14}	TD_{28}
A (Control)	7.67 ± 0.58	10.00 ± 2.00	10.33 ± 3.06
B	7.33 ± 0.58	8.33 ± 0.58	11.67 ± 2.52
C	6.67 ± 1.15	6.67 ± 0.58	7.00 ± 0.00
D	12.67 ± 0.58*	6.67 ± 1.53	9.33 ± 2.31
E	6.33 ± 0.58	5.67 ± 1.53*	10.00 ± 3.61
F	9.00 ± 1.00	4.67 ± 2.31*	7.67 ± 1.53
G	5.33 ± 0.58*	6.33 ± 0.58*	11.33 ± 1.15

5. Effect of HCQ and ivermectin administration on Lymphocyte count

TREATMENT GROUP	LYMPHOCYTE COUNT (%)		
	TD_5	TD_{14}	TD_{28}
A (Control)	86.00 ±5.20	80.00 ± 6.00	80.00 ± 6.00
B	82.00 ± 1.00	78.00 ± 2.65	77.33 ± 4.04
C	80.67 ± 2.31	84.33 ± 1.15	83.33 ± 1.15
D	70.33 ± 0.58*	85.00 ± 3.61	82.33 ± 5.03
E	82.33 ± 1.15	88.33 ± 2.08*	79.00 ± 4.00
F	80.67 ± 2.08	87.67 ± 1.53*	81.00 ± 2.00
G	83.33 ± 0.58	82.33 ± 1.53	80.67 ± 0.58

6. Effect of HCQ and ivermectin administration on Monocyte count

TREATMENT GROUP	MONOCYTE COUNT (%)		
	TD_5	TD_{14}	TD_{28}
A (Control)	8.67 ± 0.58	13.00 ± 1.00	11.00 ± 3.46
B	10.67 ± 0.58*	13.67 ± 2.31	11.33 ± 1.53
C	12.67 ± 1.15*	9.67 ± 1.53	10.00 ± 1.00
D	17.33 ± 0.58*	9.00 ± 2.00*	9.67 ± 1.53
E	11.33 ± 0.58	6.67 ± 0.58*	11.67 ± 0.58
F	10.00 ± 1.00	8.33 ± 0.58*	11.33 ± 0.58
G	10.00 ± 1.00	11.00 ± 1.00	7.00 ± 0.00*

7. Effect of HCQ and ivermectin administration on Packed Cell Volume

TREATMENT GROUP	Packed Cell Volume (%)		
	TD_5	TD_{14}	TD_{28}
A (Control)	34.33 ± 1.53	31.33 ± 3.06	32.00 ± 3.61
B	33.67 ± 0.58	33.67 ± 0.58	36.00 ± 2.00
C	24.00 ± 0.00*	36.67 ± 2.31	37.00 ± 1.00
D	28.33 ± 0.58*	38.67 ± 0.58*	33.33 ± 4.04
E	22.33 ± 0.58*	28.67 ± 4.51	35.33 ± 3.51
F	33.67 ± 1.53	31.33 ± 4.51	37.00 ± 2.00
G	20.33 ± 0.58*	33.67 ± 1.53	32.67 ± 1.53

8. Effect of HCQ and ivermectin administration on Platelet count

TREATMENT GROUP	PLATELETS (x 10^5/µL)		
	TD_5	TD_{14}	TD_{28}
A (Control)	1.35 ± 0.21	1.88 ± 0.04	3.83 ± 0.68
B	2.93 ± 0.29*	7.07 ± 0.60*	6.72 ± 0.13*
C	1.23 ± 0.18	1.82 ± 0.11	6.17 ± 0.09*
D	2.90 ± 0.07*	4.93 ± 0.63*	5.82 ± 0.10*
E	1.17 ± 0.11	2.83 ± 0.34	9.34 ± 0.15*
F	3.06 ± 0.23*	1.31 ± 0.13	6.05 ± 0.62*
G	0.42 ± 0.07*	5.48 ± 0.46*	6.46 ± 0.03*

9. Effect of HCQ and ivermectin administration on the **Mean Corpuscular Volume** (MCV)

TREATMENT GROUP	Mean Corpuscular Volume (fentoliters - fl)		
	TD_5	TD_{14}	TD_{28}
A (Control)	56.70 ± 1.14	53.67 ± 0.81	54.33 ± 1.25
B	56.27 ± 0.12	63.13 ± 17.05	60.27 ± 2.60*
C	57.60 ± 0.20	56.30 ± 2.45	57.47 ± 1.81
D	61.73 ± 0.21*	56.37 ± 0.93	55.93 ± 2.34
E	59.77 ± 0.06*	53.70 ± 1.91	57.10 ± 2.33
F	55.73 ± 0.51	53.83 ± 3.87	54.87 ± 0.25
G	60.63 ± 0.12*	53.40 ± 1.00	52.97 ± 0.40

10. Effect of HCQ and ivermectin administration on the **Mean Corpuscular Hemoglobin** (MCH)

TREATMENT GROUP	Mean Corpuscular Hemoglobin (picograms - pg)		
	TD_5	TD_{14}	TD_{28}
A (Control)	20.27 ± 0.67	21.53 ± 0.21	19.20 ± 0.20
B	20.20 ± 0.44	19.27 ± 2.35*	20.00 ± 0.50
C	20.70 ± 0.36	21.30 ± 0.46	19.20 ± 0.10
D	21.83 ± 0.35*	21.17 ± 0.12	19.90 ± 0.17
E	22.30 ± 0.30*	19.53 ± 0.35	20.30 ± 0.98
F	**20.07 ± 0.51**	**18.43 ± 0.55***	**21.80 ± 2.33***
G	20.07 ± 0.35	20.27 ± 0.06	20.50 ± 0.10

11. Effect of HCQ and ivermectin administration on the Mean Corpuseular Hemoglobin Concentration (MCHC)

TREATMENT GROUP	Mean Corpuscular Hemoglobin Concentration (g/dL)		
	TD_5	TD_{14}	TD_{28}
A (Control)	35.80 ± 1.48	39.90 ± 0.35	35.40 ± 0.36
B	35.90 ± 0.79	36.67 ± 0.55*	33.17 ± 0.57*
C	35.90 ± 0.75	37.80 ± 0.90*	34.87 ± 0.31
D	35.33 ± 0.71	37.80 ± 0.80*	35.90 ± 1.75
E	37.33 ± 0.55	36.90 ± 0.56*	35.33 ± 0.15
F	36.00 ± 0.61	36.43 ± 0.58*	36.60 ± 0.72
G	33.10 ± 0.66*	38.13 ± 0.72*	38.70 ± 0.46*

DISCUSSION

Findings of this study showed that administration of HCQ in Group C influenced hemoglobin concentration thus implying inadequate tissue oxygenation.[22,3] Iron deficiency and vitamin B-12 deficiency are believed to be the cause of the observed decreased hemoglobin concentration; as drug intake at high dosage has been proven to reduce absorption of vitamins and essential nutrients[23,24] and cause loss of appetite[25]. These two side effects of HCQ intake can result in iron and vitamin deficiency. Reduction in hemoglobin concentrations was observed upon administration of ivermectin which shows that upon administration of ivermectin, the body possibly experienced a "shock" that caused a depletion in hemoglobin concentration. The side effects of ivermectin intake such as loss of appetite, nausea, vomiting, abdominal pain, diarrhea and constipation[25] can be possible reasons for the initial shock. The depleted hemoglobin concentration equally implies reduced delivery of oxygen to cells which could be the reason behind the weakness of wistar rats to whom ivermectin was administered, in addition to weakness being a known side effect of ivermectin intake.[23] Co-administration of HCQ and Ivermectin also resulted in a decline in hemoglobin concentration. On comparing results of group E and G, it further confirms that the body possibly experienced a shock that caused a depletion in hemoglobin concentration. The further decrease in haemoglobin concentration as observed in group G when compared with group E can be explained using data from Group C which equally showed a statistically significant reduction in hemoglobin concentration. It therefore means that the low hemoglobin concentration observed in group G which is notably the lowest observed in all groups throughout the period of study is a summative effect of the co-administration of both ivermectin and HCQ on hemoglobin concentration. Side effects of ivermectin intake such as loss of appetite, nausea, vomiting, stomach pain, diarrhea and constipation[25] as well as side effects of HCQ such as low blood sugar, low amount of magnesium and potassium in the blood, anaemia, anaemia from pyruvate kinase and G6PD deficiencies[23,26] and loss of appetite[25] could be the reasons for the lower-than-normal hemoglobin concentration.[27]

Administration of HCQ and Ivermectin resulted in lower RBC counts as observed in groups C, D, E and G on TD_5. However, in all the treatment groups, RBC count was normalized by TD_{14} and TD_{28}. These findings imply a negative impact on RBC count which may be brought about either by a reduction in the synthesis of RBCs or by increasing RBC breakdown possibly caused by HCQ intake especially because anaemia is a side effect of HCQ.[28,23,24] The statistically significant increase in RBC count observed in group F on TD_5 can possibly be as a result of dehydration and somewhat related to hemoglobin concentration.

WBC count of the control group was observed to increase as the treatment days progressed. In groups C, D, E and G there was a decline in WBCs on TD_5 followed by a rapid increase on TD_{14} and then a decrease on TD_{28} (except in group E where the increase continued). It is thus postulated that low doses of HCQ in groups B and F caused a spike in WBC count within five days of drug administration indicating that HCQ at a lower concentration induced a form of stress on the system of wistar rats that resulted in elevated WBC counts possibly because of overproduction and early release of WBCs from the bone marrow. Stress of any form can result in this release of white blood cells.[29] A higher dose of HCQ in groups C and G caused a notable reduction in the WBC count within five days of drug administration. On the other hand, ivermectin administration at high and low doses caused a decrease in WBC count within the first five days of drug administration as observed in groups D and E. Notably, by TD_{28}, WBC count was regularized (similar to control) in groups B, C and F while in groups D, E and G the anomaly in WBC count when compared with control persisted.

This study's findings on Neutrophil count are expected because neutrophils make up roughly half of the white blood cell population, so it is expected that there will be an increase in neutrophil count in the event of an increase in WBC count. However, the peak neutrophil count observed in the control group is less than the normal neutrophil counts reported as

22.64 ± 6.4% for male wistar rats.[30] Since neutrophils are usually the first cells of the immune system to respond to invaders such as bacteria or viruses, it is possible that the reduced count of neutrophils recorded generally in the study (when compared with previously reported neutrophil counts) is as a result of non-stimulation of the body by the presence of bacteria and viruses.[31] In comparison with control, neutrophil counts differed statistically from control in Group D and G on TD_5 as well as groups E, F and G on TD_{14}. By TD_{28} the variations in neutrophil count across the groups were not statistically significant. This ability of neutrophil count to normalize can be hinged on the fact that drugs administered may have cleared most of the foreign bodies that can initiate neutrophil synthesis and release from the bone marrow. Also, since the neutrophil cells live for about 8 hours [31] it is easy for the body to regulate its presence in the absence of foreign bodies and organisms by reducing its release from bone marrow. There is a notable similarity in groups D and F as the wistar rats in both groups were administered ivermectin at a low dose of 0.2mg/kg body weight once daily alone (group D) and in combination with HCQ (group F). This possibly indicates that at a dose of 0.2mg/kg body weight of, ivermectin is unable to eradicate the organism elucidating the increased neutrophil synthesis. However, the presence of HCQ seems to have reduced the presence of the intruding organisms and/or effect accounting for the reduced neutrophil count in group D when compared with group F. The reduced neutrophil count observed in groups B, C, and G could be because of HCQ intake since low levels of a type of white blood cell called neutrophils is a known side effect of HCQ intake.[23,24] Findings of this study on the effects of singular and co-administration of HCQ and ivermectin on lymphocyte count showed a decline in absolute lymphocyte count or an increase in neutrophil – lymphocyte ratio, similar reports have been recorded in previous studies [30,32,33] Such findings are characteristic of disease states resulting from impairment to varying degrees by infectious diseases where the blood is in a state of hypercoagulation. [32] This finding agrees with reports from previous studies. [27,29,30] In comparison with control a statistically significant decline in lymphocyte count was observed in group D on TD_5 indicating the presence of a disease condition among the rats in this group. This conclusion is further proven by the earlier reports of increased neutrophil count in same group D on TD_5 and previously reported decline in absolute lymphocyte count in disease conditions. [32,33] Monocyte count was found to increase from TD_5 to TD_{14} followed by a decline on TD_{28} in the control group, all these values are in line with previous reports that 5% to 12% of white blood cells in the bloodstream are monocytes. [34] The monocyte count observed in this study on TD_5 in comparison with control revealed that the monocyte count in all treatment groups were higher. This finding agrees with those of WBC, neutrophil and lymphocyte count. The reduced monocyte count was statistically significant in groups D, E and F indicating the absence of foreign bodies that required clearing from the bloodstream. On TD_{28} the monocyte count was least in group G also indicating the absence of destroyed foreign bodies requiring clearance by monocytes.

Findings of this study from the analysis of blood samples revealed that the PCV of wistar rats in the control group was within the range of normal PCV value of 39.38 ± 7.6% in male rats.[30] The low hematocrit values observed in groups C, D, E and G are indicative of anaemia,[35] low red blood cell count and iron-deficient anemia that may be as a result of malnutrition[36] bearing in mind that loss of appetite which is a known side effects of HCQ intake[25] is capable of resulting in iron and vitamin deficiency as well as malnutrition. Similarly, the side effects of ivermectin such as loss of appetite[25] and anaemia could also be a cause of the observed declined PCV values. The high hematocrit value observed in group D may be indicative of dehydration. It is also possible that there may have been pulmonary conditions associated with hypoxia which may have elicited an increased production of red blood cells.[37] A previous study reported that Chloroquine and Hydroxychloroquine could result in HCQ neuromyotoxicity, proximal myopathy, neuropathy or cardiomyopathy in response to HCQ toxicity [38], these conditions could result in hypoxia.

Findings of the effects of singular HCQ and ivermectin administration as well as their co-administration on the platelet count of Wistar rats are in agreement with that of a previous study that reported normal platelet count of an adult male wistar rat as $3.44 \pm 0.8 \times 10^5/\mu L$.[30] In groups B, D and F an increase in platelet count was observed while in group G, platelet count was significantly reduced. The platelet count in Groups C and E were less than that of control, although the decrease was not statistically significant. This could mean that low doses of ivermectin and hydroxychloroquine enhanced platelet synthesis and/or release and a combination of both drugs at same dose further enhanced the synthesis and/or release while a higher concentration of ivermectin and HCQ administered reduced platelet synthesis and/or release. The far less platelet count known as thrombocytopenia observed in group G which received a combination of HCQ and ivermectin at high doses is in line with the fact that thrombocytopenia is a known side effect of taking certain medications.[39] On TD_{14} groups B and D (low dose groups) continued the trend of greatly enhanced platelet synthesis, groups C and E (high dose groups) had a slight increase in platelet count. The drug co-administered group F (low dose) had reduced platelet count while the co-administered group G had an increased platelet count. Finally, by TD_{28} all drug administered groups had significantly higher platelet counts than control implying that after long term treatment using HCQ, ivermectin or HCQ + ivermectin, there is likely to be increased blood clotting activity. It is possible that on TD_5 the body's attempt to maintain homeostasis after introduction of the drug agents at high concentration led to the consumption of platelets. Elevated platelets count known as thrombocytosis observed in all treatment groups except control may be because of inflammation and occurrence of internal minor capillary injuries occurring as a result of continuous drug intake induced toxicity. Increased platelet counts seem to have resulted from the body's attempt at enhancing its preparedness to carry out defense and improved capillary resistance to prevent RBC leakage.[39] Thrombocytosis observed in the treatment groups could also be a result of inflammation in the bowels caused by drug toxicity.

MCV of wistar rats in the control group of this study was less than the findings of past study which reported a value of 77.04 ± 20.1fl as normal for male wistar rats. Elevated levels of MCV (a measurement of the average size of RBCs)[40] were observed in group D, E and G on TD_5 and group B on TD_{28} which imply that the RBCs of rats in these groups are larger than normal. This larger than normal size could be indicative of anemia caused by vitamin B deficiency, a possible side effect of drug intake at high doses or for a prolonged period. Vitamin B deficiency induced anaemia may be resulting from malnutrition, loss of appetite or because of liver injury induced by drug toxicity. In male wistar rats, normal MCH values have been reported as 26.53 ± 4.8pg[30] which is seen to be slightly different from those observed in control in this study. In comparison with control, on TD_5, groups D and E had significantly higher MCH values indicative of anemia also caused by a vitamin B deficiency. Groups B and E had significantly less MCH than control which is indicative of iron deficiency anemia, a possible side effect of drug intake. Increased MCH values detected in group F was possibly for the same reason of vitamin B deficiency. MCHC of wistar rats reported in this study agree with a previous report of 36.69 ± 12.5g/dL for male wistar rats.[30] Lower MCHC observed in group G is indicative of iron deficiency which can be a side effect of the drug administration. All other treatment groups except group G on TD_{14} and TD_{28} in comparison with control had reduced MCHC showing that prolonged drug intake could result in anemia. On the other hand, on TD_{28}, group G had significantly higher MCHC values which may be indicative of hemolytic anemia.

CONCLUSION

HCQ and ivermectin interfere with hemoglobin concentration, WBC count, RBC count and PCV causing a decline in the values recorded when compared to control. On the other hand, platelet counts were significantly higher at low doses of the therapeutic agents but was significantly reduced when HCQ and ivermectin were co-administered at high doses insinuating the occurrence of

thrombocytopenia; a known side effect of taking certain medications.[39] Hematological indices MCV, MCH and MCHC were equally not spared from the effect of HCQ and ivermectin as differences were observed between control and the treatment groups. Most of these findings are indicative of iron deficiency anemia, vitamin B deficiency induced anemia and hemolytic anemia possibly in response to drug toxicity. The mechanism of action of both drugs on hematological parameters and indices requires attention.

Recommendations

In line with the findings from this study, the researchers recommend the following.

I.) Hydroxychloroquine and ivermectin should not be administered for a long period of time to prevent the occurrence of drug-induced toxicity.

ii.) Administration of HCQ and ivermectin should be accompanied by vitamin rich diets and intake of water to prevent vitamin deficiency and dehydration.

iii.) Continuous blood monitoring is required when administering these therapeutic agents

Acknowledgement

The authors wish to acknowledge Poiema Analytical Laboratory and Education Consult for its immense contribution towards the success of this study.

Conflict of Interests

The authors declare no conflicts of interest.

REFERENCES

1. Ujaddughe MO, Otamere HO, Olarenwaju DO, Anura A, Oriakhi O, Nwaimo VO. Correlation between Fingerprints, ABO-Blood Groups and Haemoglobin Genotype of Voluntary Blood Donors in ISTH, Irrua Edo State, Nigeria. Annals of Medical and Surgical Practice 2016; 1(1): 54-60.
2. Saravanan, M., Vidhya, K., Chavali, M., & Vaseeharan, B. (2021). Impacts of nanomaterials synthesized by greener methods on aquatic vertebrates. *Handbook of Greener Synthesis of Nanomaterials and Compounds*, 463-486.
3. MayoClinic. Complete blood count (CBC). MayoClinic News Letter [Internet]. 2023 [Cited 2023 October 15]. Available from .
4. Foley M, Tilley L. Quinoline antimalarials: mechanisms of action and resistance. Int J Parasitol. 1997; 27(2): 231-240.
5. Fox RI. Mechanism of action of hydroxychloroquine as an antirheumatic drug. Semin Arthritis Rheum 1993; **23**(2 Suppl 1): 82-91.
6. Ottesen E, Campbell W. Ivermectin in human medicine. J. Antimicrob. Chemother 1994;34: 195–203
7. WHO. Origin of COVID-19. 2019 [Cited 2023 October 18]. Available from https://www.who.int/emergencies/diseases/novel-coronavirus-2019/question-and-answers-hub/q-a-detail/coronavirus-disease-covid-19.Accessed 2023 October 18.
8. Cucinotta D, Vanelli M. WHO declares COVID-19 a pandemic. Acta Biomed. 2020;91: 157-160.
9. WHO. WHO Director-General's opening remarks at the media briefing on COVID-19. 2020 March 11 [Cited 2023 October 18]. Available from https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-1911-march-2020. Accessed 2023 October 18.
10. Patrì A, Fabbrocini G. Hydroxychloroquine and ivermectin: A synergistic combination for COVID-19 chemoprophylaxis and treatment? J Am Acad Dermatol. 2020;82(6): e221.
11. Molnar A, Lau S, Berges M, Masa RB, Solano JJ, Alter SM, Clayton LM, Shih RD, DeMets DL, Maki DG, Hennekens CH. Ivermectin in COVID-19: The Case for a Moratorium on Prescriptions. Ther Innov Regul Sci. 2022;56(3): 382-385.
12. Caly L, Druce JD, Catton MG, Jans DA, Wagstaff KM. The FDA-approved drug ivermectin inhibits the replication of SARS-CoV-2 in vitro. Antiviral Research. 2020;178:104787. doi:

10.1016/j.antiviral.2020.104787

13. Lv C, Liu W, Wang B. Ivermectin inhibits DNA polymerase UL42 of pseudorabies virus entrance into the nucleus and proliferation of the virus in vitro and vivo. Antiviral Res. 2018;159: 55–62.
14. Zhou D, Dai SM, Tong Q. COVID-19: a recommendation to examine the effect of hydroxychloroquine in preventing infection and progression. J Antimicrob Chemother. 2020; 75(7): 1667-1670.
15. Yao X, Ye F, Zhang M, Cui C, Huang B, Niu P, Liu X, Zhao L, Dong E, Song C, Zhan S, Lu R, Li H, Tan W, Liu D. In Vitro Antiviral Activity and Projection of Optimized Dosing Design of Hydroxychloroquine for the Treatment of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Clin Infect Dis. 2020 28;71(15):732-739.
16. BeMedWise. Ivermectin, the new hydroxychloroquine for COVID-19. BeMedWise [Internet]. 2021 [Cited 2023 October 14]. Available from: https://www.bemedwise.org/ivermectin/Accessed 2023 October 14. Accessed 2023 October 14.
17. Sulis G, Batomen B, Kotwani A, Pai M, Gandra S. Sales of antibiotics and hydroxychloroquine in India during the COVID-19 epidemic: An interrupted time series analysis. PLoS Med. 2021;18(7): e1003682
18. Lu D. Hydroxychloroquine sales spiked almost 100% in Australia at start of COVID pandemic study finds. The Guardian [Internet]. 2021 Oct 1 [Cited 2023 October 14]. Available from: https://www.theguardian.com/australia-news/2021/oct/01/hydroxychloroquine-sales-spiked-almost-100-in-australia-at-start-of-covid-pandemic-study-finds. Accessed 2023 October 14
19. Folorunsho-Francis A. Ivermectin disappearing from shelves, pharmacists raise alarm as demand for COVID-19 "wonder drug" soars. Punch [Internet]. 2021 [Cited 2023 October 14]. Available from https://punchg.com/ivermectin-disappearing-from-our-shelves-pharmacists-raise-alarm-as-demanmd-for-covid-19-wonder-drug-soars/. Accessed 2023 October 14.
20. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. J Pharmacol Pharmacother 2021;1(2): 87-93.
21. Enawgaw B, Alem M, Addis Z, Melku M. Determination of hematological and immunological parameters among HIV positive patients taking highly active antiretroviral treatment and treatment naïve in the antiretroviral therapy clinic of Gondar University Hospital, Gondar, Northwest Ethiopia: a comparative cross-sectional study. BMC Hematol. 2014;14(1):8.
22. Billett HH. Hemoglobin and hematocrit, In: Walker HK, Hall WD, Hurst JW, editors. Clinical Methods: The History, physical and laboratory Examinations. 3rd edition. Boston: Butterworths; 1990. Avaliable from: https://ncbi.n/n.nih gov/books/NBK259/#_ncbi_dlg_citbx_NBK259. Accessed 2023 October 16.
23. Drug Bank Online. Ivermectin. Drug Accession DB00602. Drug Bank Online [Internet]. 2023 [Cited 2023 October 17]. Available from:https://go.drugbank.com/drugs/DB00602 .Accessed 2023 October 16.
24. FDA. PLAQUENIL hydroxychloroquine sulfate tablets, USP Description. FDA [Internet] 2023 April 18, 2023 [Cited 2023 October 16]. Available from:

    https://www.accessdata.fda.gov/drugsatfda-docs/label/2017/009768s037s0471b1.pdf. Accessed 2023 October 16.
25. U.S. National Library of Medicine. Hydroxychloroquine: Medline Plus drug information. MedlinePlus [Internet]. April 18, 2023 [Cited 2023 October 16]. Available from https://medlineplus.gov/druginfo/meds/a601240.html#:~:text=Hydroxychloroquine%20is%20in20a%20class,activity%20,imunity.

Accessed 2023 October 16.

26. FDA. Ivermectin - food and drug administration. FDA [Internet] April 18, 2023 [Cited 2023 October 16]. Available from https://www.accessdata.fda.gov/drugsatfda_docs/label/2009/05742s026161.pdf.
27. MayoClinic. Hemoglobin test. MayoClinic [Internet]. 2023 [Cited 2023 October 16] Available from .
28. NHS. Red blood cell count. NHS [Internet]. 2022 [Cited 2023 October 17]. Available from https://www.ngs.uk/conditions/red-blood-couint/#:~:text=women%20usually%20have%20lower,5.2%20x%2010*12%2FL. Accessed 2023 October 17.
29. Riley LK, Rupert J. Evaluation of patient with leukocytosis. Am Fam Physician. 2015;92(11): 1004 - 1011.
30. Delwatta SL, Gunatilake M, Baumans V, Seneviratne MD, Dissanayaka MLB, Batagoda SS, Udagedara AH, Walpola P. Reference values for selected hematological, biochemical and physiological parameters of Sprague-Dawley rats at the Animal House, Faculty of Medicine, University of Colombo, Sri Lanka. Animal models and experimental medicine 2018;1(4): 250–254.
31. Mayadas TN, Cullere X, Lowell CA. The multifaceted functions of neutrophils. Annu Rev Pathol. 2014;9: 181 - 218.
32. Yuan X, Huang W, Ye B, Chen C, Huang R, Wu F, Wei Q, Zhang W, Hu J. Changes of hematological and immunological parameters in COVID-19 patients. International Journal of Hematology. 2020; 112(4): 553 – 559.
33. Awale R. B., Singh A., Mishra P., Bais P. S., Vansh K., Shamim R., Ghatak T., Hashim Z., Gupta D., Nath A., Singh R. K., Singh C. and Pande S. Routine hematology parameters in COVID-19: A predictor of disease severity and mortality. Journal of family medicine and primary care 2022;11(7): 3423 – 3429.
34. Karlmark KR, Tacke F, Dunay IR. Monocytes in health and diseases.-Minireview. Eur J Microbiol Immunol. (Bp) 2012;2(2): 97 – 102
35. Góñez C, Donayre M, Villena A, Gonzales GF. Hematocrit Levels in Children at Sea Level and at High Altitude: Effect of Adrenal Androgens. Human Biology 1993;65(1): 49 – 57.
36. Wallerstein RO. Laboratory evaluation of anemia. The Western Journal of Medicine. 1987;146 (4): 443–451.
37. Zubieta-Calleja GR, Paulev PE, Zubieta-Calleja L, Zubieta-Castillo G. Altitude adaptation through hematocrit changes. (PDF). Journal of Physiology and Pharmacology. 2007; Suppl 5 (Pt 2): 811 – 818.
38. Siddiqui AK, Huberfeld SI, Weidenheim KM, Einberg KR, Efferen LS. Hydroxychloroquine-Induced Toxic Myopathy Causing Respiratory Failure. Chest 2007; 131(2): 588-590.
39. Conley CL, Schwartz RS. Platelets (thrombocytes). Britannica [Internet]. 2023 [Cited 2023 October 16]. Available from https://www.britannica.com/science/blood-biochemistry/platelets-thrombocytes. Accessed 2023 October 16
40. Dean L. Complete blood count, Blood Groups and Red Cell Antigens. National Center for Biotechnology Information (US) [Internet]. 2005 [Cited 2023 October 16]. Available from https://www.ncbi.nlm.nih.gov/books/NBK2263/table/ch1.T1/. Accessed date 2023 October 16.