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The Effects of Frequent Therapeutic Administration of Artesunate-amodiaquine and Artemether-lumefantrine on Haematological Markers in BALB/c Mice

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Abstract

Artemisinin Combination Therapy (ACT) is readily available in malaria-endemic nations, leading to repeated drug usage by undiagnosed persons. Repeated use of ACT therapy by non-infected individuals may affect blood cells. This study explored how repeated artesunate-amodiaquine (A/A) and artemether-lumefantrine (A/L) treatment in non-infected mice affected haematological markers. 100 male BALB/c mice were randomly divided into 5 groups: non-infected and *Plasmodium berghei* NK65 infected treated with A/L and A/A 1X, 2X, 3X, 4X, 5X, and 6X, and the control group. Packed cell volume (PCV), Haemoglobin (Hb), and red blood cell (RBC) were reduced ($p>0.05$) non-significantly in the non-infected group treated with A/L or A/A six times compared to the control and infected groups. WBC rose in infected and non-infected mice treated with A/L or A/A 1X, 2X, 3X, and 6X, with a substantial rise in non-infected mice treated with A/L ($p < 0.01$) and A/A ($p < 0.001$) three times. WBC mainly rose due to lymphocytes, although neutrophils decreased. Repeated therapeutic use of A/L and A/A without infection may cause a haematological change. Continuous efforts are needed to educate the public about screening for malaria parasites before using drugs.

Keywords: Malaria; Artemether Lumefantrine; Artesunate Amodiaquine; Hematological parameters; Anemia; Neutropenia.

INTRODUCTION

Malaria is caused by *Plasmodium* parasites transmitted by female Anopheles mosquito bites (W.H.O, 2022). African countries account for 95% of worldwide malaria. Effective Malaria case management in children and adults consists of early diagnosis and fast, effective treatment with Artemisinin-based combination therapy (Koko *et al.*, 2022; W.H.O, 2022). The First-line ACT medicines for malaria in Africa are artemether-lumefantrine (A/L) and artesunate-amodiaquine (A/A), which show modest effectiveness (Zongo *et al.*, 2020; Audu *et al*., 2023).

Malaria should be screened before taking ACTs to guarantee effective therapy, but this is challenging in Africa. Malaria patients in Africa who lack parasitological confirmation of infection are routinely provided with these medications, leading to the use of ACT without being infected (Mbonye *et al*., 2010; Yeung *et al*., 2011; Cohen *et al*., 2012; Mbonye *et al*., 2013; Rusk *et al*., 2013; Idowu *et al*., 2015; Nwokolo *et al*., 2018). Multiple antimalaria dosages are typical in Nigeria due to self-prescription and free access to the drug over the counter (Owumi *et al.*, 2015).

Blood is where most antimalarial medication activities occur (Madukaku *et al.*, 2015); Long-term A/L and A/A use by non-infected people may harm blood cells. Animal tests showed that A/L at indicated doses for three days did not influence haematological parameters in non-infected rats. However, after seven days of A/L treatment, Red blood cells (RBC), Hemoglobin (Hb), and packed cell volume (PCV) dropped (Ofem *et al.*, 2013). In comparison, the study by (Adeleye *et al.*, 2012) showed that a single dose of Artesunate and A/L affected white blood cell, neutrophil, and lymphocyte counts. Long-term administration of either A/L or A/A to non-infected individuals has also been reported to have harmful effects on blood cells (Ijeomah *et al.*, 2016). Due to the current misuse of ACT in malaria-endemic regions, the goal of this experiment was to investigate the impact of repeated therapeutic administration of either A/L or A/A on the blood as further investigation is required to determine whether this substance is safe to use repeatedly. We employed a mouse model to simulate the frequent use of A/L or A/A in infected and non-infected mice. Secondly, the haematological parameters of mice were examined after one week of treatment, as

little is known about delayed or late-appearing anaemia in relation to the use of ACT for therapeutic purposes (Sowunmi *et al.*, 2017).

METHODS

Procurement of Animals and Management: 100 male adult BALB/c mice with a mean weight of 24.46± 0.07g of 8 weeks of age were used in this study. They were obtained from the University College Hospital's (UCH) Institute for Advanced Medical Research and Training in Ibadan, Nigeria. The mice were not used for any experiments before and are pathogen free. The mice were housed in plastic cages containing beddings of dried wood shavings and were fed with standard feed produced by Ladokun feed Limited, Ibadan, Oyo state, Nigeria. Mice were given constant access to food and water and were kept on a 12-hour light/12- hour dark cycle.

Research design: 100 mice were randomly allocated into 5 groups of 20 using computer-generated numbers (Johnson and Besselsen, 2002). Each group was split randomly into four replicates, each with five mice in a cage. The sample size was calculated using the equation formula (Charan and Kantharia, 2013). Group 1 consisted of mice Neither infected nor treated but given distilled water 1X, 2X, 3X, 4X, 5X and 6X times, respectively. Groups 2 are non-infected mice treated with Artemether Lumefantrine (A/L) for 1X, 2X, 3X, 4X, 5X and 6X times, respectively. While Group 3 are non-infected mice treated with Artesunate Amodiaquine (A/A) for 1X, 2X, 3X, 4X, 5X and 6X times, respectively, while Group 4 consists of mice Infected and treated with A/L for 1X, 2X, 3X, 4X. 5X and 6X times, respectively, and Group 5 mice were Infected and treated with A/A for 1X, 2X, 3X,4X, 5X and 6X times, respectively. Mice were given a week to recover after treatment or infection plus treatment before subsequent exposure. Blood was collected from 3 mice for haematological analysis one week after 1X, 2X, 3X and 6X exposure periods, in other to compare the effect of 1-3 usage with up 6 times usage of the drug on mice. The experiment was conducted following the National Institutes of Health's Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85–23, revised in 1996). The College of Veterinary Medicine research ethics committee at the Federal University of Agriculture, Abeokuta, Approved the experimental protocol (FUNAAB/COLVET/CREC/2019/07/01).

Antimalaria Drug: Artemether plus Lumefantrine (A/L) (Lumartem Anti-Malarial Tablet, 20 mg/120 mg) were obtained from Cipla Pharmaceuticals Limited in Mumbai, India, and Artesunate with Amodiaquine (A/A) (Camosunate; 100 mg/300 mg) from Geneith Pharmaceuticals Limited in Lagos, Nigeria. Treatment was carried out in all treatment groups at therapeutic doses calculated based on the manufacturer's recommendation for a man's assumed weight of 35kg. Artemether Lumefantrine was administered with a therapeutic dose of 14/6.84 mg/kg/d in six dosages at 0, 8, 24, 36, 48, and 60 hours, respectively. At the same time, Artesunate/ Amodiaquine was administered 2.86/8.58 mg/kg/d once daily for three days a week (Otuechere *et al.*, 2012)(Daikwo *et al.*, 2018). In infected groups, seven days after infection, mice were treated. Oral dosing was used to administer clinical doses using an intragastric feeding needle.

NK65 strain of *Plasmodium berghei*: *Plasmodium berghei* NK65 utilised in this study was obtained from the Chemotherapy Research Laboratory at the University College Hospital (UCH) in Ibadan, Nigeria. Microscopic slides were made from blood from the donor mice's tails to determine the parasitemia level. After the thinly diffused blood, it was air-dried, fixed, and stained with Giemsa. The parasite's presence was seen using an x100 oil immersion objective lens. We multiplied the number of parasites by the number of red blood cells (RBCs) in at least four random fields to calculate parasitemia as a percentage. Next, malaria parasite inoculums were created using blood samples from a donor mouse. Experimental mice were inoculated intraperitoneally with a low inoculum of 10^4 parasites using 0.1 ml of the donor mouse's blood. Finally, blood smears were taken, stained, and inspected under a microscope to check the establishment and monitoring of infection every other day. At the same time, parasitemia levels were fully counted on day 7^{th} post-infection and post-treatment.

Haematological Studies

The animals were treated humanely, and Blood samples were collected via the retro-orbital plexus into EDTA bottles for haematological analysis.

Packed Cell Volume (PCV): The PCV was determined by centrifuging the well-mixed anticoagulated blood sample in capillary tubes for 10 minutes at 200 revolutions per minute using a micro haematocrit centrifuge to ensure minimal cell packing. The packed Cell Volume was then calculated by measuring the height of the red cells with a PCV metre. The percentages of blood volume were used to express the values (Everds, 2007)(Cheesbrough, 2006).

Haemoglobin Measurement: The cyanmethemoglobin technique was used to determine the haemoglobin concentrations in the blood samples. The blood was diluted in a buffered solution of potassium ferricyanide and potassium cyanide to get cyanmethemoglobin. We created 1 in 25L dilutions by washing 20µl of blood in 5.0ml of modified Drabkin's fluid. Three minutes were given for complete conversion to cyanmethemoglobin. The absorbance was then measured at 540nm compared to distilled water using a Spectrophotometer (Everds, 2007)(Cheesbrough, 2006).

Red Blood Cell Counts: we made a dilution of 1 in 20 L of the anticoagulant blood sample, 0.02 ml of the blood was mixed with 4 ml Turk's solution. 0.01 ml of

the resulting mixture was inserted into the counting chamber. The red cell present in the four corners and central 1 mm^2 was counted, recorded, and the total RBC counts were calculated using formula (Everds, 2007)(Cheesbrough, 2006)

Red Cell Indices: The Mean cell Volume (MCV), Mean cell haemoglobin (MCH), and Mean Cell haemoglobin concentration (MCHC) were estimated using the formula described by (Everds, 2007)(Cheesbrough, 2006)

Mean Cell Haemoglobin (MCH): This is the quantity of haemoglobin (in pictogram form) contained in an average red cell. The value was derived using the haemoglobin concentration and red blood cell numbers formula.

$$MCH = (Hb \times 10) / (\text{Total RBC}) \text{ (Pg or } 10^{-12}/g)$$

Mean Cell Volume (MCV): This is the average volume of red blood cells measured in femtoliters. The MCV was calculated from the PCV and red cell using the formula.

$$MCV = (PCV \times 10) / \text{Total RBC (fl or } 10^{-15}/L)$$

Mean Cell Haemoglobin Concentration (MCHC): This is the quantity of haemoglobin in 100ml of packed red blood cells instead of the amount of haemoglobin in whole blood. The MCHC was computed from the haemoglobin and PCV represented as g/dl,

$$MCHC = (Hb \times 100) / PCV \text{ (g/dl)}$$

White Blood Cell Counts: The blood sample was diluted by washing 50µl of blood into 950µl of the diluting fluid to give a final dilution of 1 in 20. The dilution was then mixed and loaded into the counting chamber. The white cells present in the four corners 1 mm^2 areas were counted. The final White Cell Count for the whole blood sample was calculated using (Everds, 2007)(Cheesbrough, 2006).

White blood Cell Differentials: Leishmann staining method was used to determine the percentage of each type of WBC, prepared slides were viewed under the microscope X100 oil immersion objective, and the cells were counted and recorded (Everds, 2007)(Cheesbrough, 2006).

Statistical analysis: Raw data from the laboratory were analysed, and graphs were plotted using GraphPad Prism 8.0 computer program. To determine the Significant difference between the various treated group and the control, a One-way analysis of variance (ANOVA) was used. The tables provided the findings as mean ± standard Error mean (SEM) (n =3). Shapiro-Wilk tests were first used to determine whether the data's distribution was normal. The significance of mean values *, ** and *** indicate significant differences at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively compared to the respective control groups.

RESULTS

Mean Parasitemia levels at day seven post-infection were less than 5% in mice infected in each consecutive time of 1X, 2X, 3X, 4X, 5X and 6X, respectively. Day 7 post-treatment with A/L or A/A for 1X, 2X, 3X, 4X, 5X and 6X, respectively, after the corresponding infection recorded 0% parasitemia.

Effects of Repeated treatment of Non-infected and Infected mice with either A/L or A/A on PCV, Hb, RBC and WBC.

To ascertain the level of haematological damage of Non-infected and Infected groups treated repeatedly with either AL or AA, the blood PCV, Hb, RBC, WBC, RBC indices, and differential WBC count were analysed.

Non-infected and Infected mice exposed to A/L and A/A 1X, 2X, 3X and 6X didn't significantly alter ($p>0.05$) the level of blood PCV (Fig. 1a), Hb (Fig. 1b), and RBC (Fig.2a) respectively when compared with their controls. Although there was a non-significant ($p>0.05$) decrease in PCV, Hb, and RBC levels in the non-infected group treated with A/L only once compared to the control 1X and the rest group 1X, this reduction was not later observed after treatment for 2X and 3X period. Also, mice infected and treated with A/L 3X recorded non-significant ($p>0.05$) reduced PCV, Hb and RBC compared to the control group 3X, and the rest group treated 3X; this reduction was not later seen after 6X treatment (Fig. 1 a, b and 2a). The non-infected groups treated with A/L and A/A for 6X non-significantly ($p>0.05$) reduced the PCV, Hb and RBC levels compared to the control group (Fig. 1a,1b and 2a). The MCV, MCH and MCHC of Non-infected and Infected mice treated with either A/L or A/A for 1X, 2X and 3X were not altered compared to the control group. The non-infected group treated with A/L for 6X significantly ($p < 0.05$) reduced the MCV value compared to the control, the Infected treated with A/L or A/A 6X and the non-infected treated with A/A 6X (Table 1).

The WBC counts were lower in control compared to the non-infected and Infected groups treated with either A/L or A/A 1X, 2X, 3X, and 6X, except group (INF+AA), treated 6X (Fig. 2B). However, WBC counts increased in the non-infected groups treated with either A/L or A/A 1X and 3X following an increasing trend with a significant increase in group AL ($p < 0.01$) and AA ($p < 0.001$) for 3X but dropped after treatment for 6X (Fig. 2b).

Exposure of either A/L or A/A 1X, 2X, 3X and 6X to non-infected and infected groups reduced the neutrophil level and increased the lymphocyte level compared to the control (Table 2). The eosinophils level was significantly higher in non-infected mice treated with either A/L for

2X and 3X compared with the control, following an increasing trend with a Significant highest increase in 2X ($p < 0.05$) and 3X ($p < 0.001$), after which it drops in treatment for 6X. Infected mice treated with either AL or AA 6X recorded higher eosinophil levels than the non-infected and control groups (Table 2). Basophil level was higher in non-infected and infected groups treated with either A/L or A/A for 2X, 3X, and 6X compared to the control, with a Significant ($p < 0.001$) increase in the non-infected group treated with A/A for 1X and 6X and Infected group treated with A/A for 1X, 3X and 6X times. Exposure of the Non-infected group to either A/L or A/A 1X, 2X and 3X times and only A/A for 6X increased the Monocyte level compared to the control group. In contrast, the infected group treated with either A/L or A/A 1X and 2X, and only A/A for 3X and 6X increased Mono level compared to the control group (Table 2).

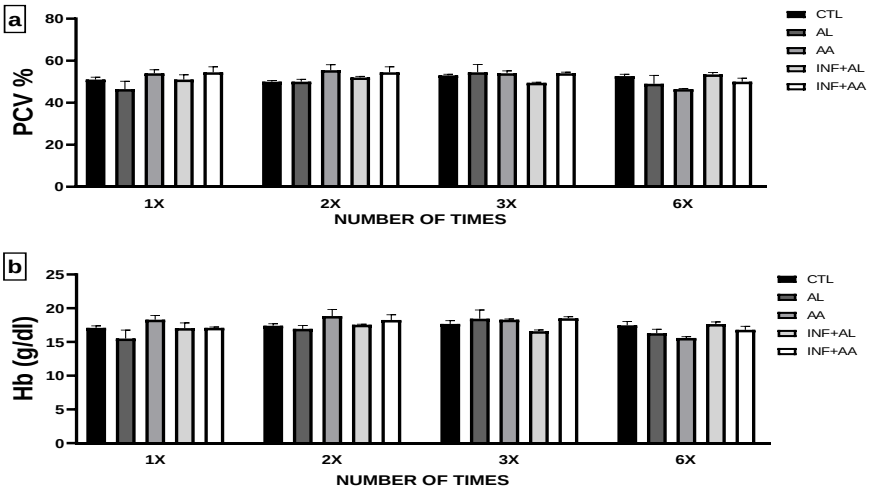

Figure 1. (A)PCV level (B)Hb level: of Infected and Non-infected mice treated with either A/L or A/A regime 1X, 2X, 3X and 6X times. PCV: Packed Cell Volume; Hb: Haemoglobin AL; Treatment of Non-Infected with A/L therapeutic doses; AA: Treatment of Non-Infected with A/A therapeutic doses; INF+AL: Infected with *P. berghei* and treated with A/L therapeutic; INF+AA: Infected with *P. berghei* and treated with A/A therapeutic; CTL: Control; 1X: One time; 2X: Two times; 3X: Three times; 6X: Six times. Values are expressed as mean ± SEM (n = 3). *, **, and *** indicate significant differences at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively compared to its corresponding control groups.

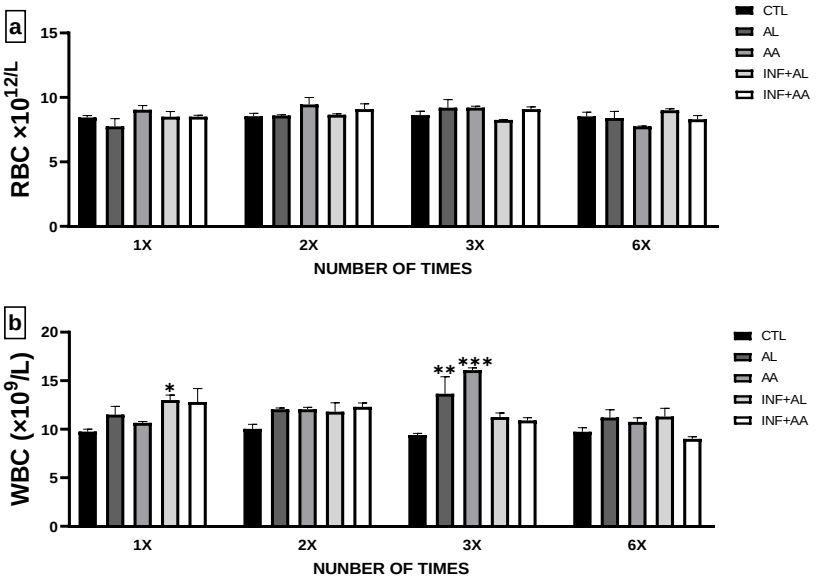

Figure 2. (A) RBC level (B) WBC level: of Infected and Non-infected mice treated with either A/L or A/A regime for 1X,2X, 3X and 6X times. AL: RBC: Red Blood Cell; WBC: White Blood Cell. Treatment of Non-Infected with A/L therapeutic doses; AA: Treatment of Non-Infected with A/A therapeutic doses; INF+AL: Infected with *P. berghei* and treated with A/L therapeutic; INF+AA: Infected with *P. berghei* and treated with A/A therapeutic; CTL: Control; 1X: One time; 2X: Two times; 3X: Three times; 6X: Six times. Values are expressed as mean ± SEM (n = 3). *, **, and *** indicate significant differences at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively compared to its corresponding control groups.

Table 1. Effect of Repeated Usage of AL and AA On the Level Of MCV, MCH And MCHC Blood Parameters In Mice.

No. of Times of Infection and/or Treatment	Infection and/or Treatment	RED BLOOD CELL INDICES		
		MCV (Fl)	MCH (pg)	MCHC (g/dl)
1X	**CTL**	60.35±0.33	20.24±0.00	33.54±0.19
	AL	59.95±0.14	20.00±0.06	33.35±0.03
	AA	59.70±0.17	20.20±0.00	33.85±0.09
	INF+AL	60.05±0.14	20.05±0.03	33.45±0.03
	INF+AA	60.00±0.12	20.15±0.14	33.55±0.14
2x	**CTL**	58.60±0.91	20.42±0.82	34.82±0.94
	AL	58.15±0.95	19.70±0.46	33.85±0.20
	AA	58.85±0.66	19.95±0.14	33.95±0.14
	INF+AL	60.10±0.06	20.30±0.12	33.75±0.20
	INF+AA	59.85±0.20	20.05±0.03	33.50±0.17
3x	**CTL**	61.14±2.03	20.53±1.11	33.54±0.79
	AL	59.25±0.03	20.05±0.03	33.85±0.03
	AA	58.70±0.29	19.90±0.17	33.90±0.23
	INF+AL	60.00±0.12	20.15±0.14	33.55±0.14
	INF+AA	59.30±0.69	20.30±0.17	34.30±0.23
6X	**CTL**	61.84±1.18	20.50±0.61	32.37±0.92
	AL	58.30±0.06*	19.40±0.40	33.30±0.23
	AA	60.00±0.17	20.15±0.14	33.55±0.14
	INF+AL	59.45±0.20	19.60±0.12	33.00±0.06
	INF+AA	60.25±0.03	20.25±0.09	33.60±0.12

MCV: Mean Cell Volume; MCH: Mean Cell Haemoglobin; MCHC: Mean Cell Haemoglobin; AL: Treatment of Non-Infected with A/L therapeutic doses; AA: Treatment of Non-Infected with A/A therapeutic doses; INF+AL: Infected with *P. berghei* and treated with A/L therapeutic; INF+AA: Infected with *P. berghei* and treated with A/A therapeutic; CTL: Control; 1X: One times; 2X: Two times; 3X: Three times; 6X: Six times. Values are expressed as mean ± SEM (n = 3). *, **, and *** indicate significant differences at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively compared to its corresponding control groups.

Table 2. Effect of repeated usage of AL and AA on WBC differentials in mice granulocytes.

No. of Times of Infection and/or Treatment	Infection and Treatment	WHITE BLOOD DIFFERENTIALS				
		NEUTROPHILS (%)	LYMPHOCYTES (%)	EOSINOPHILS (%)	BASOPHILS (%)	MONOCYTES (%)
1X	**CTL**	36.00±1.73	62.33±2.03	1.00±0.00	0.00±0.00	0.67±0.33
	AL	26.00±0.58***	71.00± 0.58**	1.50±0.29	0.00±0.00	1.00±0.00*
	AA	25.50±0.29***	71.00± 0.57**	0.50±0.29	1.50±0.29***	1.50±0.29
	INF+AL	32.00±2.31	64.00± 2.31	2.00±0.00*	1.00±0.00	1.00±0.00
	INF+AA	29.00±0.58**	67.00± 1.16	1.50±0.29	1.50±0.29***	1.00±0.00
2x	**CTL**	36.67±0.33	61.00±0.58	1.00±0.00	0.33±0.33	0.00±0.00
	AL	28.50±0.87***	67.50±0.29*	2.00±0.58*	1.00±0.00	1.00±0.00**
	AA	28.00±0.58***	69.50±0.29**	1.00±0.00	0.50±0.29	1.00±0.00**
	INF+AL	27.50±0.29***	69.50± 0.29**	0.50±0.29	0.50±0.29	1.00±0.00**
	INF+AA	26.50±0.29***	70.50±0.29***	1.00±0.00	0.50±0.29	1.50±0.29***
3x	**CTL**	38.67±0.67	60.00±0.58	1.00±0.00	0.00±0.00	0.33±0.33
	AL	28.00±0.58***	66.50± 0.88*	3.50±0.29***	1.00±0.00	1.00±0.00
	AA	32.00±0.58*	63.00± 1.15	2.00±0.00*	1.00±0.00	2.00±0.00***
	INF+AL	29.00±2.31***	69.50± 2.02***	1.00±0.00	0.50±0.29	0.00±0.00
	INF+AA	27.00±0.58***	67.00± 1.73*	3.00±0.00***	2.00±0.00***	1.00±0.00
6X	**CTL**	36.67±2.33	61.00±1.73	1.00±0.00	0.00±0.00	1.00±0.00
	AL	27.00±0.58***	69.00±0.58**	1.00±0.00	1.00±0.00	1.00±0.00
	AA	29.50±2.60**	66.00± 3.46	1.00±0.00	1.50±0.29***	1.50±0.29*
	INF+AL	30.00±1.73*	66.50±2.02	1.50±0.29	0.50±0.29	1.00±0.00
	INF+AA	30.50±0.29*	64.00± 0.58	2.00±0.00*	2.00±0.58***	1.67±0.33**

AL: Treatment of Non-Infected with A/L therapeutic doses; AA: Treatment of Non-Infected with A/A therapeutic doses; INF+AL: Infected with *P. berghei* and treated with A/L therapeutic; INF+AA: Infected with *P. berghei* and treated with A/A therapeutic; CTL: Control; 1X: One time; 2X: Two times; 3X: Three times; 6X: Six times. Values are expressed as mean ± SEM (n = 3). *, **, and *** indicate significant differences at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively compared to its corresponding control groups.

DISCUSSION

In this study, A/L and A/A were very effective against the malaria parasite. Both drugs are seen to clear the parasite after 7-day post-treatment during each repeated infection and treatment. Reports have shown that both A/L and A/A have high malaria treatment success (Davlantes *et al.*, 2018; Marwa *et al*., 2022). Although, several reports have shown that malaria cause anaemia and changes in other haematological variables (Osaro *et al.*, 2014; Omarine Nlinwe and Nange, 2020; Audu *et al*., 2021), and recurring malaria attacks can lead to life-threatening anaemia (Bakhubaira, 2013; Dhangadamajhi *et al.*, 2019). However, this present study was designed to determine if repeated therapeutic exposure to A/L and A/A when not infected could affect blood cell counts and predispose animals to anaemia, as very little was found in the literature on this question. This investigation shows that the PCV, Hb and RBC were not significantly altered by the therapeutic use of A/L and A/A for 1X, 2X, 3X and 6X in non-infected mice compared to the control groups. This outcome is contrary to the previous study, which has shown acute haemolytic anaemia following the use of artemisinin (Rehman *et al.*, 2014). Furthermore, a study by (Geerligs *et al.*, 2003) corroborates that malaria chemoprophylaxis improves mean haemoglobin levels. While the term "haematocrit conservation" was spawned because it was seen that there is little to no drop in haematocrit following ACTs, even when parasitaemia are heavy (Gbotosho *et al.*, 2014; Sowunmi *et al*., 2017). These findings suggest that taking this drug for 1X, 2X and 3X, and 6X therapeutically when not infected would not significantly alter the PCV, Hb and RBC after one week of each repeated treatment.

Prolonged repeated treatment of non-infected mice with either A/L or AA for up to six consecutive times reduced the levels of PVC, Hb, and RBC insignificantly compared to the control and infected groups. It indicated that the prolonged use of this treatment in the absence of infection could potentially modify the haematological parameter. Evidence suggests that exposure to either AL or AA over an extended period can have a detrimental effect on haematological markers (Ofem *et al*., 2013; Ijeomah *et al.*, 2016). Although the use of A/L 1X when non-infected and when infected 3X insignificantly also reduced the PCV, Hb and RBC value, although there was a recovery after subsequent treatment, this shows that the use of A/L over A/A could potentially also alter the PCV, Hb and RBC parameters when used.

(White, 2018) reported that being infected and treated with artemisinin is seen to cause haematolytic anaemia after 1-3 weeks of drug usage. What is surprising in this experiment is that repeated infecting mice and treatment with either A/L or A/L 1X, 2X, 3X and 6X didn't alter the PCV, Hb and RBC. These results reflect those of (Sowunmi *et al*., 2017), who also found that both A/A and A/L have been observed to dramatically lower the prevalence of anaemia in younger and older children who have malaria after therapy (Sowunmi *et al.*, 2017). This result supports that repeated malaria infection and treatment with therapeutic doses of either A/L or A/A would improve the haematological parameters.

MCV, MCH and MCHC are indices of erythrocyte shape, size, and haemoglobin content changes. Studies have shown that Artesunate does not affect the total RBC, MCH, and MCHC levels (Bigoniya *et al.*, 2015)(Ijeomah *et al.*, 2016). The current study found that the MCV, MCH, and MCHC values obtained after treatment of Non-infected and Infected groups with either A/L or A/A for 1X, 2X and 3X were not significantly different compared to the control group. Implies that the red blood cells are normal in size and concentration after treatment of Infected and Non-infected with the drugs for 1X, 2X and 3X. Although after repeated treatment with therapeutic dosages of A/L for 6X to non-infected mice, the MCV value was significantly reduced; this indicates that using A/L repeatedly without being infected could decrease the average size of the red blood cells in mice.

White blood cells in the body provide a unique defence system against infections and hazardous substances. In this study, apart from the group repeatedly infected and treated with A/A 6X, there was an increase in WBC count in both Infected and Non-infected mice treated with either AL or AA therapeutic doses for 1X, 2X, 3X and 6X times. The increase was due to a rise in Lymphocytes, eosinophils, basophils, and monocytes, but a decrease in neutrophil was observed in the treated groups. The increase in WBC and lymphocyte counts suggests an immunological response induced by the drug as they are mobile components of the body's defence systems. As (Adeleye *et al.*, 2012) Found that A/L can raise total WBC counts and lymphocyte counts while decreasing neutrophil counts, which they ascribed to the immunological response caused by the medication. In our study, we noticed either A/L or A/A usage once or repeatedly resulted in a significant increase in the WBC of Non-infected groups. However, a study by (Ijeomah *et al.*, 2016) found a substantial drop in WBC after long-term A/L and A/A usage; also, in this present study, Non-infected mice treated with AL and AA for three consecutive times had the highest increase in WBC count but dropped after six consecutive times. This result has shown that usage of AL and AA could increase the WBC count when used repeatedly but could drop after prolonged usage.

In malaria-infected individuals, counts of white blood cells (WBCs), neutrophils, monocytes, lymphocytes, and eosinophils were considerably reduced (Kotepui *et al.*, 2014). In this study, repeated usage of AL and AA in infected and non-infected mice for 1,2,3 and 6 consecutive times had a significant reduction in neutrophil, with a substantial decrease in non-infected

mice treated with either A/L or A/A for 1 and 6 times compared to the control and infected group. This result implies that treatment with either A/L or A/A without Infection could lead to Neutropenia. Low neutrophil has been associated with intermittent (weekly) doses of amodiaquine for malaria prevention. (Zwang *et al.*, 2012). This study reported a significant increase in eosinophil, basophil, and monocyte in groups treated with AL and AA; this increase may be due to responses of the Antimalaria drugs (Adeleye *et al.*, 2012; Bigoniya *et al*., 2015). The limitation of this study lies in the fact that the study did not include taking blood immediately after treatment as a subgroup for comparison. Notwithstanding these limitations, the study still shows us the extent of haematological alteration caused when these drugs are taken repeatedly.

CONCLUSIONS

The result of this study shows that the use of A/L and A/A therapeutically in a repeated manner without being infected could result in haematological alteration. This study lays the groundwork for future clinical and further animal work on this subject. Further research could address more longer-time effects of taking this drug and check the haematological parameter immediately after each treatment. In addition, more significant efforts are needed to enlighten the public on the need to repeatedly screen for malaria parasites before every repeated use of Antimalarial drugs.

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***Authors' Contributions*:** AD came up with the experiment's idea. AD, IBA, IOA, and MFM designed the research methodology and carried out the experiments. PVB provided technical assistance. AD worked on the draft manuscript; the final version was read, edited, and approved by all authors.

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