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The effects Artesunate Amodiaquine and Artemether Lumefantrine on some Hematological Parameters in Healthy Male Albino Rats

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Abstract
The effects of oral administration two artemisinin-based combination therapies in wistar albino rats on haematological parameters in albino rats were investigated. Thirty two albino rats were divided into seven groups. Group 1 (control) received distilled water, Group 2 received 1.43 mg / 3.86 mg/kg body weight of artesunate amodiaquine (AA), Group 3 received 2.8 mg / 7.7 mg/kg body weight of AA, Group 4 received 5.71 mg / 15.14 mg/kg body weight of AA, Group 5 received 0.57 mg / 3.43 mg/kg body weight of artemether lumefantrine (AL), Group 6 received 1.14 mg/ 6.86 mg/kg body weight of AL and Group 7 received 2.28 mg / 13.72 mg/kg body weight of AL. The different dosages of the drugs were administered orally for 18 days. Result showed that the drugs significantly ($p<0.05$) decreased white blood cell count in all treated groups, but a decrease in haemoglobin concentration which was only significant ($p<0.05$) with the group treated 5.71 mg / 15.14 mg/kg AA. Red blood cell count decreased in groups treated with 1.43 mg / 3.86 mg AA, 0.57 mg / 3.43 mg AL, 1.14 mg/ 6.86 mg AL and 2.28 mg / 13.72 mg AL and this decrease was significant ($p<0.05$) only for the group treated with 2.28 mg / 13.72 mg AL. There was a non-significant ($p>0.05$) increase in hematocrit for groups III and IV but a non-significant ($p>0.05$) decrease was observed for groups VI and VII. Red cell indices were observed to decrease but non-significantly except red cell distribution width which was observed to increase significantly. Granulocyte and medium size cell distribution levels showed significant decrease in all treated groups, lymphocyte also decreased significantly in groups 4, 5 and 6 while percentage lymphocyte increased significantly in groups 2, 5, 6 and 7 and granulocytes percent showed significant decrease in groups. Platelet count increased in groups 3 and 6, platelet total volume increased significantly in groups 3 and 4 while mean platelet volume and platelet distribution width count increased only in groups 3 and 7. These results suggest that long term administration of both drugs variable effects mainly mild to moderate adverse effects on the haematological parameters in albino rats.

Key words: *Malaria, Artesunate amodiaquine, Artemether lumefantrine, Haemoglobin, WBC and RBC.*

INTRODUCTION

Malaria is one of the most important infectious diseases worldwide. Malaria is caused by the parasite plasmodium (P), which has different species including *P. falciparium, P. vivax, P. ovale,* and *P. malariae*. It is perhaps the world's most devastating human parasitic infection (Nicholas and Breman, 2005; Udeme and Omotayo, 2012) and Africa bears the greatest burden of this disease. Efforts to control the spread of this disease have been largely unsuccessful (Rulisa *et al.,* 2007). Nigeria is one of the countries most affected by malaria and accounts for 25 percent of global malaria cases. The treatment of malaria has faced several challenges due to the development of drug resistance by the parasite (Obianime and Aprioku, 2009), cost and adverse effects of antimalarial agents (Bloland *et al*., 2000).

Artemisinin-based combination therapies (ACTs) are still the first-line treatments of *Plasmodium falciparum* malaria globally; they are more effective than non-artemisinin–based

combination or monotherapies, and they reduce the chances of development of drug resistance in *P. falciparum* (Adjuik *et al.,* 2004; Gbotosho *et al.,* 2011; Ewenighi *et al.,* 2013). This has therefore necessitated the use of combined therapy of artemisinin with other antimalarial agents known as the artemisinin-based combination treatments (ACTs).

Haematologic changes associated with malaria infection are well recognized, specific changes may vary with the level of malaria endemicity, nutritional status and demographic factors and malaria immunity (Sumbele *et al.,* 2010). Scientific data on the adverse effects of these combination therapies on haematological parameters are still very scanty. This research was designed to investigate the long term administration effects of artesunate amodiaquine (AA) and artemether lumefantrine (AL) on haematological parameters in albino rats.

MATERIALS AND METHOD

Materials

Drugs

The study was conducted using ACTs; Artesunate Amodiaquine (100 / 270 mg) manufactured by Maphar (Sanofi Aventis) laboratories 20250 Casablanca, Morocco and Coartem norvatis or Arthemeter Lumefantrine (20/120mg) by Novartis Pharmaceuticals Corporation Suffern, New York, U.S.A were purchased from Ben-Jonalson Pharmacy Keffi, Nasarawa State. They were kept at room temperature until used.

Experimental animals

Thirty two (32) healthy adult albino rats of average weight (50-100 g) were obtained from accredited National Veterinary Research Institute (NVRI), Vom, Jos-plateau state. The rats were weighed, marked and grouped into seven groups with five rats in each group. They were housed in clean well ventilated cages and fed with vital feed and given water *ad libitum* for two weeks to acclimatize them to laboratory condition. The study lasted for five weeks. The principles governing the care of laboratory animals as laid out by the Department of Zoology, Nasarawa State University Keffi were duly observed.

Methods

Preparation of Drugs

One tablet (100/270)mg of Artesunate Amodiaquine was dissolved in 35ml of distilled water and this was vigorously shaken for proper dissolution. 20/120mg Arthermether lumefanthrine was dissolved in 17.5ml of distilled water with vigorous shaking for proper dissolution. Volumes corresponding to dose calculated for each rat were taken out of this stock and administered to the rats orally.

Experimental design

The rats were given two different types of drugs (AL and AA) base on their body weight.

Group 1: (control) receives distilled water.
Group 2: received 1.43 mg / 3.86 mg/kg body weight of artesunate amodiaquine.
Group 3: received 2.8 mg / 7.7 mg/kg body weight of artesunate amodiaquine.
Group 4: received 5.71 mg / 15 mg/kg body weight of artesunate amodiaquine.
Group 5: received 0.57 mg / 3.43 mg/kg body weight of artemether lumefantrine.
Group 6: received 1.14 mg / 6.86 mg/kg body weight of artemether lumefantrine.
Group 7: received 2.28 mg / 13.72 mg/kg body weight of artemether lumefantrine.

Groups 2&5; 3&6; and 4&7 were half the therapeutic dose, therapeutic dose and 2x the therapeutic dose respectively. The reconstituted drugs as described above were administered to the animals orally for 18 days based on their body weight basis.

Sample collection

The rats were anaesthetized with chloroform and 5ml of blood was collected from the rats through cardiac puncture and dispensed into well-labelled EDTA containers to avoid coagulation.

Haematological assays (full blood count)

Whole blood was used to determine the full blood count parameters and all the analysis were performed using standard diagnostic Diatro reagents (Diatro-Lyse-Diff, Diatro Cleaner, Diatro Diluent reagents) using Abacus junior haematology analyzer (Diatron.GmbH.Wein Austria).

Weight gain

$$\text{Weight gain} = \frac{\text{Weight after experiment-Weight before acclimatization}}{\text{Weight after experiment}}$$

Statistical Analysis

The results were statistically analyzed using one way analysis of variance (ANOVA). Data are expressed as mean ± standard deviation (mean ± SD) of triplicate determinations.

RESULTS

Table 1 indicated that the rats gained weight progressively. It was observed that the weight increased significantly in weight ($p< 0.05$) before experiment in groups 3 and 7 which were treated with 2.8mg/7.7 mg of AA and 2.28 mg/13.72 mg of AL respectively. At week one and two there was also significant ($p< 0.05$) increase in the weight of all groups except in group 5 which received 1.14mg / 6.86mg of AL when compared to the control.

Table 1: Weekly Body weight changes of the experimental animals

Group	Treatment/kg B.Wt	WBACT (g)	WBEXPT (g)	WAWK1 (g)	WAWK2 (g)	WAEXP (g)	WG (g)
I	Control	86.38±12.56	114.39±13.22	146.02±20.33	188.29±24.43	188.29±24.43	101.91±28.70
II	1.43mg/3.86mgAA	43.88±1.42*	77.92±4.70	93.95±8.92*	115.75±6.91*	115.75±6.91*	71.87±6.14*
III	2.8mg/7.7mgAA	45.55±12.91*	92.34±6.060	114.38±8.34*	142.30±6.75*	142.30±6.75*	87.00±5.46
IV	5.71mg/15mgAA	69.80±6.93*	93.49±8.74	106.34±10.67*	122.81±19.01*	122.80±19.01*	53.00±22.10*
V	0.57mg/3.43mgAL	81.07±15.07	110.75±11.63	127.42±12.88*	141.47±12.32*	141.47±12.32*	60.40±2.83*
VI	1.14mg/6.86mgAL	34.86±2.35*	59.66±6.18*	78.42±7.01*	99.14±9.12*	99.14±9.12*	64.28±9.08*
VII	2.28mg/13.72mgAL	25.63±6.47*	51.83±13.18*	75.17±16.54*	92.13±22.90*	92.13±22.90*	65.41±21.65*

Values are expressed as Mean ± SD of replicate determinations, the superscript * shows that there is a significant difference with the control at $p<0.05$. Where WBEXPT= weight before experiment, WAWK1= weight at week one of experiment, WAWK2= weight at week two of experiment, WAEXP= weight on the last day of experiment, WG= weight gained

Table 2 showed a non-significant ($p>0.05$) decrease in HGB for all the groups except in group VII (treated with 2.28 mg/13.72 mg of AL) when compared with the control. There was a non-significant ($p>0.05$) increase in RBC for groups III and IV but a decrease was observed in

groups II, V, VI and VII. The decrease in RBC was however, significant ($p<0.05$) in group VII. A significant ($p<0.05$) decrease was observed in the WBC of all the treated groups.

An increase in % HCT was observed in groups III and IV treated with AA but this increase was not statistically significant ($p > 0.05$) when compared with the control group. A non-significant ($p>0.05$) decrease was however observed in groups (VI and VII) treated with AL but the decrease was also not statistically significant at ($p > 0.05$) when compared with the control group.

Table 2: Effect artesunate/amodiaquine and Artemether/lumefantrine on white blood cell count (WBC), haematocrit concentration (HCT), Red blood cell count (RBC), and haemoglobin concentration (HGB) of healthy albino rats.

Group	Treatment/ kg B.Wt	HGB (g/dl)	RBC (10^6/Ul)	HCT (%)	WBC (10^6)/Ul)	RBC:WBC
I	Control	11.78±1.06	7.51±0.54	37.55±2.4	15.39±4.89	51.77±12.49
II	1.43 mg / 3.86 mg of AA	11.24±1.24	7.05±1.04	37.33±5.54	5.57±0.97*	130.48±34.73*
III	2.8 mg / 7.7 mg of AA	11.68±0.87	7.79±0.54	39.61±3.53	5.81±1.05*	153.26±30.78*
IV	5.71 mg / 15 mg of AA	11.75±0.26	7.52±0.58	40.20±3.93	5.01±0.69*	138.37±30.47*
V	0.57 mg / 3.43 mg of AL	11.13±0.46	7.15±0.40	38.24±3.74	3.28±0.77*	231.39±76.73*
VI	1.14 mg / 6.86 mg of AL	11.23±0.84	7.13±0.73	35.44±2.45	3.20±0.71*	236.92±85.97*
VII	2.28 mg / 13.72 mg of AL	10.00±0.85*	6.34±0.35*	34.00±1.87	4.50±0.55*	142.74±20.17*

Values are expressed as Mean ± SD of replicate determinations, *there is a significant difference with the control at $p<0.05$. Where HGB=haemoglobin concentration, RBC= red blood cell count, HCT= hematocrit concentration, WBC = white blood cell count, RBC: WBC = red blood cell to white blood cell ratio.

Table 3 showed a non-significant ($p>0.05$) increase in MCV when compared with the control for all treated groups except for group VI which is non-significantly ($p>0.05$) lower than the control group. MCH increased non-significantly ($p>0.5$) except in groups IV and V which showed a non-significant ($p>0.5$) decrease when compared with the control group. All groups showed a non-significant ($p>0.5$) decrease in MCHC except in group VI which is non-significantly ($p>0.5$) higher when compared with the control group. Furthermore the RDWC values showed significant ($p < 0.05$) increase in groups 2, 3, 4 and 7 when compared with the control group.

Table 3: Effect of artesunate/amodiaquine and Artemether/lumefantrine on Red cell indices and Red cell distribution width concentration (RDWC) in healthy albino rats.

Group	Treatment/ kg B.Wt	MCV (fl)	MCH (pg)	MCHC (g/dl)	RDWC (%)
I	Control	50.00±1.00	15.64±0.47	31.30±1.07	18.54±1.52
II	1.43 mg / 3.86 mg of AA	53.00±2.80	16.02±1.35	30.32±3.10	21.60±1.62*
III	2.8 mg / 7.7 mg of AA	53.50±3.32	15.70±1.58	29.45±3.11	23.35±1.68*
IV	5.71 mg / 15 mg of AA	51.00±2.55	14.98±0.69	29.54±1.62	22.96±1.80*
V	0.57 mg / 3.43 mg of AL	53.50±5.45	15.63±1.04	29.60±3.70	20.60±1.47
VI	1.14 mg / 6.86 mg of AL	49.75±2.06	15.80±0.55	31.73±1.23	17.93±0.71
VII	2.28 mg / 13.72 mg of AL	53.60±2.19	15.80±0.98	29.42±1.08	20.60±1.68*

Values are expressed as Mean ± SD of replicate determinations, *there is a significant difference with the control at $p<0.05$.Where MCH = mean cell haemoglobin, MCH= mean cell volume, MCHC = mean cell haemoglobin concentration, RDWC% = Erythrocyte distribution index concentration/red cell distribution width

In table 4, there is a decrease in lymphocytes (LYM) levels but is only significant ($p<0.05$) in groups IV, V, and VI when compared to the control group. The levels of MID and GRA decreased significantly ($p< 0.05$) in all treated groups when compared with the control. LY% increased significantly ($p< 0.05$) in groups II, V, VI, and VII except in group III. However, group IV showed a non-significant ($p>0.05$) decrease when compared with the control. MI % decreased non-significantly ($p>0.05$) for all the groups except group V which showed a non-significant ($p>0.05$) increase when compared with the control. GR% increased significantly ($p<0.05$) in all the groups treated with AL. It increased in AA treated (group IV) though the increase was not significant ($p>0.05$) when compared with the control.

Table 4: Effect of artesunate/amodiaquine and Artemether/lumefantrine on white blood cell indices in healthy albino rats.

GROUP	Treatment/kg B.Wt	LYM (10^3/Ul)	MID (10^3/Ul)	GRA (10^3/Ul)	LY (%)	MI (%)	GR (%)
I	Control	3.49±1.12	1.99±1.17	4.84±0.53	36.38±5.7	16.90±6.72	48.06±5.41
II	1.43 mg / 3.86 mg AA	2.39±0.10	1.06±0.17*	1.61±0.53*	50.62±9.07*	18.62±3.68	40.06±10.39
III	2.8 mg / 7.7 mg AA	2.74±1.56	0.99±0.55*	1.92±0.21*	46.56±16.03	17.08±7.49	36.35±13.76*
IV	5.71 mg / 15 mg AA	1.59±0.50*	0.88±0.48*	3.15±0.19*	34.80±12.01	18.62±3.98	57.02±4.76
V	0.57 mg / 3.43 mg AL	1.60±0.38*	0.69±0.27*	1.27±0.21*	54.65±2.50*	20.83±4.99	29.75±10.76*
VI	1.14 mg / 6.86 mg AL	1.81±0.42*	0.53±0.09*	0.87±0.18*	56.65±6.50*	16.75±1.78	30.50±3.81*
VII	2.28 mg / 13.72 mg AL	3.13±1.48	0.86±0.29*	0.87±0.22*	60.76±11.62 *	17.58±5.72	29.68±5.32*

Values are expressed as Mean ± SD of replicate determinations, *there is a significant difference with the control at p<0.05.Where LYM = lymphocyte count, MID= medium size cell distribution count, GRA= granulocyte count, LY% = Percentage lymphocyte, MI% = percentage medium size cell distribution count, GR% = percentage granulocyte.

In table 5 all the treated groups showed an increase in platelet count (PLT) except in group VII. The increase in PLT observed was only significant in group IV and VI when compared with the control.

Only groups 3 and 4 a significant increase ($p< 0.05$) in percentage platelet (PCT %) when compared to the control. There is also an increase in mean platelet volume (MPV) and platelet distribution width count (PDWC) in all treated groups but it was significant ($p< 0.05$) only in groups 3 and 7 when compared to the control group.

DISCUSSION

Some of the antimalarial agents had been reported to have some adverse effect on body functions (Raji *et al.,* 2005; Obianime and Aprioku, 2009). Data from this investigation showed that Artesunate/Amodiaquine and Artemether/lumefantrine have effects on the haematological parameters in albino rats of Wistar strain. The effects were variable. In some they showed an increased as seen in PLT level, while in others they decreased as is the case of WBC in all treated groups. The haematological analysis was aimed to provide useful information on the general state of the blood after administration of the two ACTS.

The most widely used criteria for the toxic action of a drug in animals are reduction in body weight gain, detection of biochemical, physiological and histological abnormalities in the vital organs.

Table 5: Platelet indices responses of albino rats after ingestion of artesunate amodiaquine (AA) and artemether lumefantrine (AL) after 18 days.

Group	Treatment/ kg B.Wt	PLT (10^3/Ul)	PCT (%)	MPV (fl)	PDWC
I	Control	500.40±43.11	0.52±0.11	8.90±0.46	33.10±1.00
II	1.43 mg / 3.86 mg of AA	576.60±127.48	16.02±1.35	30.32±3.10	35.84±1.74
III	2.8 mg / 7.7 mg of AA	635.25±83.70	0.59±0.10*	9.08±0.71*	36.83±3.70
IV	5.71 mg / 15 mg of AA	645.20±134.68*	0.56±0.10*	8.70±1.04	34.22±3.20
V	0.57 mg / 3.43 mg of AL	554.50±87.60	0.54±0.13	9.00±0.41	34.98±2.50
VI	1.14 mg / 6.86 mg of AL	674.25±133.58*	0.53±0.18	8.60±0.22	34.55±0.93
VII	2.28 mg / 13.72 mg of AL	460.60±44.77	0.43±0.11	9.92±0.77*	38.06±1.35*

Values are expressed as Mean ± SD of replicate determinations, *there is a significant difference with the control at $p<0.05$. Where PLT = platelet concentration, PCT= platelet total volume, MPV = mean platelet volume concentration, PDWC = Platelet Distribution width count.

In the present study the gain in body weight in the experimental animals suggests no gross toxicity of the drug. Previous studies showed a similar trend adding that intake of nutrients and water are essential to the physiological status of the animals and to the accomplishment of the proper response to the drug tested, instead of a "false" response due to improper nutritional conditions (Stevens and Mylecraine, 1994; Iversen and Nicolaysen, 2003). It was observed that administration of the drugs did not interfere with the diet pattern and water consumption in the experimental rats throughout the period of the study. This must have brought about the increase in weight observed in the experimental animals.

The significant decrease in WBC observed in all treated groups suggests leukopenia which can suppress the immune system when the drug is taken for a long period of time, leading to increased risk of infection (Abbas and Lichtman, 2003). White blood cells in the body constitute a special system for combating infections and toxic agents (Adeleye *et al.,* 2012). They are the mobile units of the body's protective systems. Any increase in WBC and lymphocyte counts is suggestive of an immunological response induced by the drug.

The decrease in red blood cell and haemoglobin concentration though not statistically significant is suggestive of anemic conditions. Preclinical data suggested that repeated exposure to certain antimalaria drug may affect blood cell counts and predispose to anemia (Obianime and Aprioku, 2009; Obianime *et al*, 2011). The MCV and MCH are indicators to indicate variations in erythrocyte shape, size and hemoglobin content. The MCV, MCH and MCHC values obtained in this study were not significant, meaning that the red blood cells are normal in size and concentration, thus suggesting normocytic and normochromic of the red blood cell. Also no significant difference was recorded in MCH which indicates that the red cells are normochromic. Increase in RDW_C may be as a result of abnormal shape of the RBC, the large size of immature cells causes variation (Bernard *et al.,* 2006).

The significant decrease in white blood cell indices including lymphocyte count medium size cell distribution count, granulocyte and percentage granulocyte observed may be due to long

term administration of the drug. Lymphocytes play a role in the antibody humoral and cell mediated immunity dependent cellular toxicity to invade pathogens in the body (Brooks *et al.,* 2001) and a decrease in lymphocyte concentration is associated with increased rates of infection after trauma (Abbas and Lichtman, 2003).

The significant increase observed in PLT levels in some groups and PCT levels in some groups is suggestive of thrombocytosis (also known as thrombocythaemia), a medical term for high platelet count. Long term administration of the drugs might have led to the observed increase in PLT and PCT. Production of excess platelets, may lead to sticking together of the platelets which will result to clotting. This can lead to increase in the chance of having stroke or heart attack (Abbas and Lichtman, 2003). MPV and PDWc are useful in the differential diagnosis of thrombocytopenia, and it is also used to differentiate between reactive thrombocytosis and thrombocytosis associated with myeloproliferative disorder. The increase observed in MPV and PDWc in some groups may be due to reactive thrombocytosis induced by the drug.

In conclusion, artesunate/amodiaquine and artemether/lumefantrine have effect on hematological parameters in experimental animals. Increase in platelet indices which indicate thrombocytosis, the decrease observed in white blood cell suggestive of an adverse immunological response induced by the drug. All these evidences indicate that the long term administration of both drugs have potential to produce toxic effect on haematological parameters.

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