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A COMPARATIVE STUDY ON THE EFFICACY OF ARTEMISININ MONOTHERAPY AND COMBINATION THERAPY TO *PLASMODIUM BERGHEI* IN EXPERIMENTAL MICE

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ABSTRACT

The objective of the present study was to evaluate the comparative efficacy of different dosage regimens of arteether and artemether-lumefantrine to *Plasmodium berghei* induced haematological changes. Our secondary objective was to identify parasite clearance time (PCT) after treatment. Chloroquine sensitive *P. berghei* ANKA strain parasites were infected to albino mice through intraperitoneal route. α, β-arteether and artemether-lumefantrine in sub-therapeutic, therapeutic and double-therapeutic doses were taken for comparative efficacy study. PCT was determined by taking peripheral blood smear up to day 28 and haematological parameters were estimated by following standard protocols. Double therapeutic dose of artemether-lumefantrine has low parasite clearance time 48 h (2 days) with haematological recovery. Artemether-lumefantrine is a highly efficacious combination with rapid parasite clearance and with low parasite burden. But it is evident that arteether is on the edge of developing resistance.

KEY WORDS: *Plasmodium berghei*, α, β-arteether, artemether-lumefantrine, parasite clearance time, haematological parameters.

INTRODUCTION

Malaria is an acute, recurrent and sometimes chronic vector borne disease which has worldwide distribution in tropical and subtropical regions. In spite of worldwide efforts to reduce malaria transmission, it is still the major cause of morbidity and mortality. Malaria is the most lethal parasitic disease in the world, annually affecting approximately 500 million people mostly in African sub-Saharan countries.[1,2]

Malaria infection in humans and animals is caused by the parasite Plasmodium. Several species of Plasmodium have the ability to cause malaria in animals, including rodents. *Plasmodium berghei* is a unicellular protozoan, originates from the forests of Central Africa where its natural hosts are thicket rat *Grammonys surdaster* and the mosquito *Anopheles dureni*. *P. berghei* infections are lethal to laboratory rodents.

Like all malaria parasites of mammals, including the four human malaria parasites, *P. berghei* is transmitted by Anopheles mosquitoes and it infects the liver after being injected in to blood stream by a bite of an infected female mosquito. After few days of development and multiplication, these parasites leave the liver and invade erythrocytes. The multiplication of the parasite in the blood causes the pathology such as anaemia and damage to essential organs of the host such as lungs, liver and spleen.

In recent years, malaria has become more difficult to control and treat because malaria parasites have become resistant to drugs and mosquitoes that transmit the disease have become resistant to insecticides. There is a changing trend in the management malaria and combined drug therapy is fast replacing mono-therapeutic approaches in the management of the diseases.

α, β-Arteether (30:70 mixture) is oil soluble ethyl ether derivative of artemisinin with longer $t_{1/2}$ and more lipophilic properties which favours its accumulation in brain tissue for the treatment of cerebral malaria.[3] The co-formulation of an artemisinin derivative combined with another antimalarial drug was designed to improve efficacy, compliance and minimize selection of drug-resistant parasite strains.[4] Artemether was used in this study, because it exerts rapid schizontocidal effect, combined with lumefantrine, which has a much longer half-life, producing a low recrudescence rate.[5]

Our primary objective was to evaluate comparative efficacy of different dosage regimens of arteether

(artemisinin monotherapy) and artemether-lumefantrine (artemisinin combination therapy) on *P. berghei* induced haematological changes. Our secondary objective was to identify parasite clearance after treatment with artemisinin derivatives that might provide a simple indicator that could be used to screen for artemisinin resistance during *in vivo* efficacy studies.

MATERIALS AND METHODS

Animals

Swiss albino male mice of 18-22 g weight were selected as experimental animals. Animals were allowed to acclimatize for one week before initiation of experiment. They were housed in plastic cages with rice husk as beddings provided with access to clean drinking water *ad libitum*. The animals were handled in accordance with the guidelines in the Guide of the Care and Use of Laboratory Animals.[6] Animal experiments were designated and approved by Institutional Animal Ethical Committee of ANU College of Pharmacy, Guntur, Andhra Pradesh.

Parasite

Chloroquine sensitive *P. berghei* ANKA strain parasites were maintained by weekly passage of infected blood in albino mice. A standard inoculum consisting of 1.0×10^7 parasitized erythrocytes was prepared from the infected donor mice with >25% parasitemia.

Inoculation of experimental animals

Parasitized red blood cells used for inoculation were obtained by cardiac puncture from a donor mouse. The infected blood was collected in an anticoagulant and diluted to the desired density in 0.9% normal saline. Each mouse was inoculated with 1.0×10^7 parasitized red blood cells of *P. berghei* suspension. The infection of the recipient mice were initiated by needle passage of the parasite preparation from the donor to healthy test animals via the intraperitoneal route as described previously.[7] The day of inoculation was defined as day 0 and subsequent days as day 1, day 2, and day 3 up to day 28.

Drugs

A six-dose regimen of artemether (20 mg) co-formulated with lumefantrine (120 mg) tablets (LUMERAX-20 DT) from IPCA Laboratories Limited, India). Arteether (E-Mal from Themis Chemicals Limited, Mumbai, India) injection. 1 ampoule of E-mal contains 150 mg of drug given intramuscularly for 3 days. Artemether-lumefantrine tablets were powdered in a glass mortar, mixed with distilled water to provide the weight-adjusted dose and administered as aqueous suspensions by oral gavage. Artemether-lumefantrine and arteether doses were given at 0, 8, 24, 36, 48 and 60 h. On first day drug was given in a double divided dose with a time interval of 8 h. Drug dosages were according to the body weight of mouse.

Distribution of animals

Each group contains 5 animals. Initially all the experimental animals were infected with 1.0×10^7 parasitized red blood cells intraperitoneally.

Group 1 (Inf): In this group, animals were only infected with *P. berghei* parasites.
Group 2 (Inf + DT. AE 1): In this group, animals were infected with *P. berghei* and then treated with sub-therapeutic dose of arteether.
Group 3 (Inf + DT. AE 2): This group, initially infected with *P. berghei* parasites and then treated with therapeutic dose of arteether, 150 mg/kg for 3 days administered intramuscularly.
Group 4 (Inf + DT. AE 3): This group, of animals initially parasitized and the treated with double-therapeutic dose of arteether.
Group 5 (Inf + DT. AL 1): In this group animals were infected with *P. berghei* and then treated with sub-therapeutic dose of artemether-lumefantrine.
Group 6 (Inf + DT. AL 2): This group initially infected and then treated with therapeutic dose of artemether-lumefantrine, 3.5 mg/kg body weight through oral gavage for 3 days.
Group 7 (Inf + DT. AL 3): This group of animals initially parasitized and then treated with double-therapeutic dose of artemether-lumefantrine.

In every group parasitemia was determined by peripheral blood smears. At the end of the treatment, the mice were euthanized with chloroform as anaesthesia and blood sample was collected through cardiac puncture in sterile labelled EDTA coated tubes for haematological investigation.

Course of infection of *P. berghei* in experimental animals

Thin blood films were prepared on clean slides, initially fixed with methanol. A large drop of blood is put at the centre of a clean dry slide. The drop is spread with an applicator stick, then the smear is thoroughly dried in a horizontal position. Blood smears were stained with Giemsa for 5-8 min. Subsequently, distilled water was poured on the surface of the smears to remove excess stain and then dried. A field was selected using x10 objective where the RBCs are in an evenly distributed monolayer followed by the x100 oil immersion objective. A minimum of 1000 RBCs were counted and among them number of infected RBCs will be recorded daily throughout the study period. The percent of infected RBCs (parasitemia) was determined by enumerating the number of infected RBCs in relation to the number of uninfected RBCs[8] as follows.

$$\text{Percentage of Parasitemia} = \frac{\text{No.of Infected RBCs}}{\text{No.of RBCs counted}} \times 100.$$

Determination of responses of infection to treatment

Thin peripheral blood smears were prepared every day after the appearance of parasites. Occurrence of clearance of parasites following treatment was determined in each animal as parasite clearance time (PCT). Parasite clearance time was defined as the time after drug administration until there was no patent parasitemia for at least 48 h. The time taken for the parasites to reappear in the animals during 28 days of follow up was defined as recrudescence time.

Estimation of haematological parameters: In all experimental groups whole blood was drawn to see the frequency of haematological abnormalities especially anaemia, thrombocytopenia and reduced blood counts during malaria. Haemoglobin (Hb) by Sahli's haemoglobinometric method; red blood cells, white blood cells and platelets were estimated estimated by new improved Neubauer chamber method; differential leucocyte count (DLC) by smear;[9,10] packed cell volume (PCV) by micro haematocrit method. All other erythrocytic indices like mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) was calculated from the haematological data obtained as follows.

$$MCV = \frac{PCV}{RBC} \times 10$$

$$MCH = \frac{Hb}{RBC} \times 10$$

$$MCHC = \frac{Hb}{PCV} \times 100$$

STATISTICAL ANALYSIS

Results of individual parameters were expressed as mean ± standard deviation. The comparison between the experimental groups was performed by student t-test in MINITAB 11. 12. 32. Bit statistical package and graphs were drawn from MS Excel. The values are statistically significant at $p < 0.05$*

RESULTS

Percentage of parasitemia in *P. berghei* infected group

Course of infection in *P. berghei* infected mice without treatment was evaluated. Infection was given on day 0. Then the parasitemia became noticeable on 3rd day (72 hours) after inoculation and followed an acute course. On day 3 initial parasitemia was 15% reaching peak parasitemia of 38% on day 6 and then mortality was observed on day 8.

Parasite clearance time in sub-therapeutic dose of arteether treated group

Parasites were appeared on day 3 with 21% after inoculation. On day 3 sub-therapeutic dose of arteether was administered intramuscularly for 3 days. Parasite biomass increased gradually reaching peak level on day 8 with 31%. Then parasites gradually reduced and completely disappeared in peripheral blood smear on day 12 with 0%. PCT in sub-therapeutic dose of arteether was 240 h (10 days). No recrudescence was observed during follow up (Figure 1).

Parasite clearance time in therapeutic dose of arteether treated group

Initial parasitemia was 19% on day 3. On day 3 therapeutic dose of arteether was given intramuscularly. Parasitemia reached peak level on day 6 with 30%. Then the parasites were decreased gradually on day 7 with 28% and on day 8 with 24%, then completely disappeared on day 9. No recrudescence was observed during follow up. Therapeutic dose of arteether PCT was 168 h (7days) (Figure 2).

Parasite clearance time in double-therapeutic dose of arteether treated group

Parasites become visible in peripheral blood smear on day 3 with 18%. On day 3 double-therapeutic dose of arteether was given for 3 days. Parasites rose to peak on day 4 with 21%. Then parasites were reduced and disappeared on day 7 with 0%. No recrudescence was observed during follow up. PCT for double-therapeutic dose of arteether was 120 h (5 days) (Figure 3).

Parasite clearance time in sub-therapeutic dose of artemether-lumefantrine treated group

Initial parasitemia was 20% on day 3. Sub-therapeutic dose of artemether-lumfantrine was given orally for 3 days. Peak parasitemia was observed on day 5 with 28%. Parasites disappeared on day 7. No recrudescence was observed during follow up. PCT in sub-therapeutic dose of artemether-lumefantrine was 120 h (5 days) (Figure 4).

Parasite clearance time in therapeutic dose of artemether-lumefantrine treated group

Initial parasitemia was 18% on day 3. On day 3 therapeutic dose of artemether-lumefantrine was administered for 3 days. On day 4 parasites reached peak with 20%. Parasites disappeared on day 5. No recrudescence was observed during follow up. The PCT for therapeutic dose of artemether-lumefantrine was 72 h (3 days) (Figure 5).

Parasite clearance time in double-therapeutic dose of artemether-lumefantrine treated group

Initial parasitemia was 16% on day 3. Double-therapeutic dose of artemether-lumefantrine was given for 3 days orally. Parasites disappeared on day 4 with 0%. No recrudescence was observed during follow up. PCT for double-therapeutic dose of artemether-lumefantrine was 48 h (2 days) (Figure 6).

Variations in haematological parameters in different experimental groups

The Table 1, represents that Hb, RBC and PCV were significantly decreased; MCV and platelets were decreased significantly ($p < 0.05$); MCH and MCHC were elevated significantly; WBC and neutrophils were

significantly increased ($p < 0.05$); basophils, monocytes, eosinophils and lymphocytes were decreased significantly in infected mice.

Table 2, reports the changes in haematological parameters between infected and sub-therapeutic dose of arteether, Hb, PCV and MCV increased significantly when compared to infected group. RBC, MCH and MCHC were reduced significantly, platelets were increased significantly and WBC count reduced significantly ($p < 0.05$). In DLC, neutrophil count reduced significantly. Basophil count increased, monocyte count is same as in infected, eosinophil and lymphocyte counts were elevated significantly.

Therapeutic dose of arteether, Hb, RBC, PCV and MCV values were raised significantly ($p < 0.05$) when compared to infected mice. MCH and MCHC concentrations were lowered significantly ($p < 0.05$). Platelet count was raised significantly ($p < 0.05$). WBC count was reduced significantly ($p < 0.05$). In DLC, neutrophil count decreased significantly ($p < 0.05$) indicating neutropenia. Basophils reduced ($p < 0.05$) and eosinophils and lymphocytes elevated significantly (Table 3).

Table 4, illustrate the change between infected and double-therapeutic dose of arteether, Hb, RBC, PCV and MCV values were elevated significantly ($p < 0.05$) when compared to infected mice. MCH and MCHC were reduced significantly ($p < 0.05$). Platelets were enhanced significantly ($p < 0.05$). WBC count was reduced ($p < 0.05$) compared to infected group. In DLC, basophils, monocytes, eosinophils and lymphocyte counts were increased significantly while neutrophil count decreased significantly ($p < 0.05$).

Changes in haematological values between infected group and sub-therapeutic dose of artemether-lumefantrine treated group are represented in Table 5. Hb, RBC, PCV and MCV values were increased significantly ($p < 0.05$) when compared to infected group. MCH and MCHC were decreased significantly. Platelets were elevated significantly when compared to infected group ($p < 0.05$). WBC count was reduced significantly ($p < 0.05$). In DLC, neutrophil count was reduced ($p < 0.05$), basophils increased, monocytes increased slightly, eosinophil and lymphocytes were increased significantly ($p < 0.05$).

Table 6, denotes variation between infected group and artemether-lumefantrine therapeutic dose administered group. Hb, RBC, PCV and MCV increased significantly ($p < 0.05$). MCH and MCHC were reduced significantly ($p < 0.05$). Platelet count was raised significantly ($p < 0.05$). WBC count decreased significantly ($p < 0.05$). In DLC, neutrophil count decreased. Basophils and eosinophil counts were elevated but not significantly. Monocytes and lymphocyte count were increased significantly ($p < 0.05$).

Table 7, depicts variations between infected and double-therapeutic dose of artemether-lumefantrine administered mice. Hb nearly reached to control, significantly increased ($p < 0.05$) when compared to infected group. RBC, PCV, MCV were increased significantly when compared to infected mice and nearly reached to control. MCH and MCHC values were reduced significantly when compared to infected group. Platelets were significantly increased ($p < 0.05$) and nearly reached to control. WBC was significantly reduced but slightly more than control. In DLC; neutrophil count declined, basophils elevated significantly, monocytes enhanced significantly ($p < 0.05$) which was equal to control, eosinophil count was increased significantly and lymphocytes amplified significantly ($p < 0.05$) when compared to infected group but more than control values.

Table 1: Changes in haematological values between control and *P. berghei* infected experimental mice.

Parameter	Control (n=5)	Infected (n=5)	P-value
Hb (g/dL)	14.2±0.138	9.7±0.191	0.0000*
RBC count (10^6/mm^3)	8.2±0.020	6.9±0.148	0.0001*
PCV (%)	43.6±0.071	24.5±0.402	0.0000*
MCV(fL)	54.5±0.351	46.9±0.152	0.0000*
MCH (pg)	15.5±0.141	18.0±0.051	0.0000*
MCHC (g/dL)	27.4±0.134	36.9±0.568	0.0000*
PLT count (10^3/mm^3)	946.1±0.148	543.6±0.444	0.0000*
WBC count (cells/mm^3)	4.8±0.018	5.97±0.084	0.0000*
Neutrophils (%)	16.3±0.022	37.7±0.195	0.0000*
Basophils (%)	0.11±0.016	0.04±0.016	0.0002*
Monocytes (%)	1.8±0.016	1.7±0.018	0.0000*
Eosinophils (%)	1.13±0.025	1.03±0.023	0.0004*
Lymphocytes (%)	80.6±0.164	59.5±0.355	0.0000*

The values are given as mean ± SD, *statistically significant at $p < 0.05$,
Hb: haemoglobin, RBC count: red blood cell count, PCV: packed cell volume, MCV: mean cell volume, MCH: mean cell haemoglobin, MCHC: mean cell haemoglobin concentration, PLT count: platelet count, WBC count: white blood cell count.

Table 2: Changes in haematological values between infected group and sub-therapeutic dose of arteether drug treated group.

Parameter	Infected (n=5)	Inf+ DT.AE 1 (n=5)	P-value
Hb (g/dL)	9.7±0.191	13.7±0.023	0.0000*
RBC count (10^6/mm^3)	6.9±0.148	7.3±0.114	0.0082^{NS}
PCV (%)	24.5±0.402	38.4±0.019	0.0000*
MCV(fL)	46.9±0.152	50.1±0.187	0.0000*
MCH (pg)	18.0±0.051	15.0±0.228	0.0000*
MCHC (g/dL)	36.9±0.568	28.4±0.019	0.0000*
PLT count (10^3/mm^3)	543.6±0.444	940.1±0.241	0.0000*
WBC count (cells/mm^3)	5.97±0.084	3.8±0.041	0.0000*
Neutrophils (%)	37.7±0.195	13.8±0.018	0.0000*
Basophils (%)	0.04±0.016	0.09±0.002	0.0036*
Monocytes (%)	1.7±0.018	1.7±0.008	0.0001*
Eosinophils (%)	1.03±0.023	1.08±0.001	0.010*
Lymphocytes (%)	59.5±0.355	83.2±0.023	0.0000*

The values are given as mean ± SD, *statistically significant at $p < 0.05$, NS: not significant,
Hb: haemoglobin, RBC count: red blood cell count, PCV: packed cell volume, MCV: mean cell volume, MCH: mean cell haemoglobin, MCHC: mean cell haemoglobin concentration, PLT count: platelet count, WBC count: white blood cell count.

Table 3: Changes in haematological values between infected group and therapeutic dose of arteether drug treated group.

Parameter	Infected (n=5)	Inf+ DT.AE 2 (n=5)	P-value
Hb (g/dL)	9.7±0.191	13.9±0.038	0.0000*
RBC count(10^6/mm^3)	6.9±0.148	7.6±0.020	0.0007*
PCV (%)	24.5±0.402	39.8±0.02	0.0000*
MCV(fL)	46.9±0.152	47.9±0.027	0.0001*
MCH (pg)	18.0±0.051	15.0±0.048	0.0000*
MCHC (g/dL)	36.9±0.568	26.4±0.018	0.0000*
PLT count (10^3/mm^3)	543.6±0.444	938.2±0.783	0.0000*
WBC count(cells/mm^3)	5.97±0.084	3.93±0.028	0.0000*
Neutrophils (%)	37.7±0.195	13.8±0.023	0.0000*
Basophils (%)	0.04±0.016	0.01±0.002	0.010*
Monocytes (%)	1.7±0.018	1.8±0.025	0.0011*
Eosinophils (%)	1.03±0.023	1.09±0.002	0.0051*
Lymphocytes (%)	59.5±0.355	79.9±0.108	0.0000*

The values are given as mean ± SD, *statistically significant at $p < 0.05$,
Hb: haemoglobin, RBC count: red blood cell count, PCV: packed cell volume, MCV: mean cell volume, MCH: mean cell haemoglobin, MCHC: mean cell haemoglobin concentration, PLT count: platelet count, WBC count: white blood cell count.

Table 4: Changes in haematological values between infected group and double-therapeutic dose of arteether drug treated group.

Parameter	Infected (n=5)	Inf+ DT.AE 3 (n=5)	P-value
Hb (g/dL)	9.7±0.191	14.1±0.200	0.0000*
RBC count (10^6/mm^3)	6.9±0.148	7.8±0.025	0.0002*
PCV (%)	24.5±0.402	41.5±0.023	0.0000*
MCV(fL)	46.9±0.152	50.3±0.230	0.0000*
MCH (pg)	18.0±0.051	15.3±0.187	0.0000*
MCHC (g/dL)	36.9±0.568	29.3±0.270	0.0000*
PLT count (10^3/mm^3)	543.6±0.444	940.1±0.230	0.0000*
WBC count (cells/mm^3)	5.97±0.084	4.9±0.0259	0.0000*
Neutrophils (%)	37.7±0.195	9.9±0.019	0.0000*
Basophils (%)	0.04±0.016	0.18±0.007	0.0000*

Monocytes (%)	1.7±0.018	1.8±0.015	0.0000*
Eosinophils (%)	1.03±0.023	1.11±0.016	0.0003*
Lymphocytes (%)	59.5±0.355	87±0.339	0.0000*

The values are given as mean ± SD, *statistically significant at $p < 0.05$,
Hb: haemoglobin, RBC count: red blood cell count, PCV: packed cell volume, MCV: mean cell volume, MCH: mean cell haemoglobin, MCHC: mean cell haemoglobin concentration, PLT count: platelet count, WBC count: white blood cell count.

Table 5: Changes in haematological values between infected group and sub-therapeutic dose of artemether-lumefantrine drug-treated group.

Parameter	Infected (n=5)	Inf+ DT.AL 1 (n=5)	P-value
Hb (g/dL)	9.7±0.191	14±0.110	0.0000*
RBC count (10^6/mm^3)	6.9±0.148	7.9±0.048	0.0002*
PCV (%)	24.5±0.402	40.1±0.228	0.0000*
MCV(fL)	46.9±0.152	51.4±0.192	0.0000*
MCH (pg)	18.0±0.051	15.1±0.270	0.0000*
MCHC (g/dL)	36.9±0.568	29.9±0.083	0.0000*
PLT count (10^3/mm^3)	543.6±0.444	949.4±1.34	0.0000*
WBC count (cells/mm^3)	5.97±0.084	4.2±0.207	0.0000*
Neutrophils (%)	37.7±0.195	13.2±0.195	0.0000*
Basophils (%)	0.04±0.016	0.4±0.013	0.0000*
Monocytes (%)	1.7±0.018	1.8±0.016	0.0000*
Eosinophils (%)	1.03±0.023	1.1±0.022	0.0005*
Lymphocytes (%)	59.5±0.355	83.4±0.141	0.0000*

The values are given as mean ± SD, *statistically significant at $p < 0.05$,
Hb: haemoglobin, RBC count: red blood cell count, PCV: packed cell volume, MCV: mean cell volume, MCH: mean cell haemoglobin, MCHC: mean cell haemoglobin concentration, PLT count: platelet count, WBC count: white blood cell count.

Table 6: Changes in haematological values between infected group and therapeutic dose of artemether-lumefntrine drug treated group.

Parameter	Infected (n=5)	Inf+ DT.AL 2 (n=5)	P-value
Hb (g/dL)	9.7±0.191	14.0±0.114	0.0000*
RBC count (10^6/mm^3)	6.9±0.148	8.2±0.021	0.0000*
PCV (%)	24.5±0.402	42.4±0.08	0.0000*
MCV(fL)	46.9±0.152	53.7±0.217	0.0000*
MCH (pg)	18.0±0.051	14.6±0.036	0.0000*
MCHC (g/dL)	36.9±0.568	26.5±0.0239	0.0000*
PLT count (10^3/mm^3)	543.6±0.444	950.1±0.200	0.0000*
WBC count (cells/mm^3)	5.97±0.084	4.8±0.022	0.0000*
Neutrophils (%)	37.7±0.195	13.3±0.020	0.0000*
Basophils (%)	0.04±0.016	0.03±0.002	0.12^{NS}
Monocytes (%)	1.7±0.018	1.8±0.011	0.0000*
Eosinophils (%)	1.03±0.023	1.3±0.352	0.11^{NS}
Lymphocytes (%)	59.5±0.355	82.5±0.023	0.0000*

The values are given as mean ± SD, *statistically significant at $p < 0.05$, NS: not significant,
Hb: haemoglobin, RBC count: red blood cell count, PCV: packed cell volume, MCV: mean cell volume, MCH: mean cell haemoglobin, MCHC: mean cell haemoglobin concentration, PLT count: platelet count, WBC count: white blood cell count.

Table 7: Changes in haematological values between infected group and double-therapeutic dose of artemether-lumefantrine drug treated group.

Parameter	Infected (n=5)	Inf+ DT.AL 3 (n=5)	P-value
Hb (g/dL)	9.7±0.191	14.3±0.019	0.0000*
RBC count (10^6/mm^3)	6.9±0.148	8.3±0.024	0.0000*
PCV (%)	24.5±0.402	44.8±0.016	0.0000*
MCV(fL)	46.9±0.152	54.7±0.024	0.0000*
MCH (pg)	18.0±0.051	15.6±0.022	0.0000*
MCHC (g/dL)	36.9±0.568	30.1±0.191	0.0000*
PLT count (10^3/mm^3)	543.6±0.444	950.2±0.380	0.0000*
WBC count (cells/mm^3)	5.97±0.084	5.8±0.021	0.024*
Neutrophils (%)	37.7±0.195	8.0±0.059	0.0000*
Basophils (%)	0.04±0.016	0.09±0.001	0.0039*
Monocytes (%)	1.7±0.018	1.8±0.025	0.0011*
Eosinophils (%)	1.03±0.023	1.1±0.011	0.0013*
Lymphocytes (%)	59.5±0.355	89±0.138	0.0000*

The values are given as mean ± SD, *statistically significant at $p < 0.05$, NS: not significant,
Hb: haemoglobin, RBC count: red blood cell count, PCV: packed cell volume, MCV: mean cell volume, MCH: mean cell haemoglobin, MCHC: mean cell haemoglobin concentration, PLT count: platelet count, WBC count: white blood cell count.

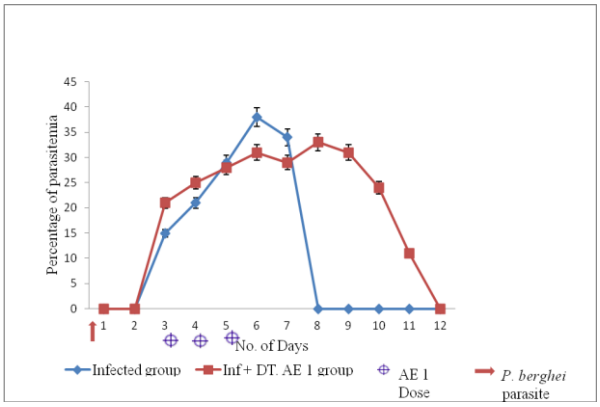

Fig. 1: Effect of sub-therapeutic dose of arteether to *P. berghei* infected mice.

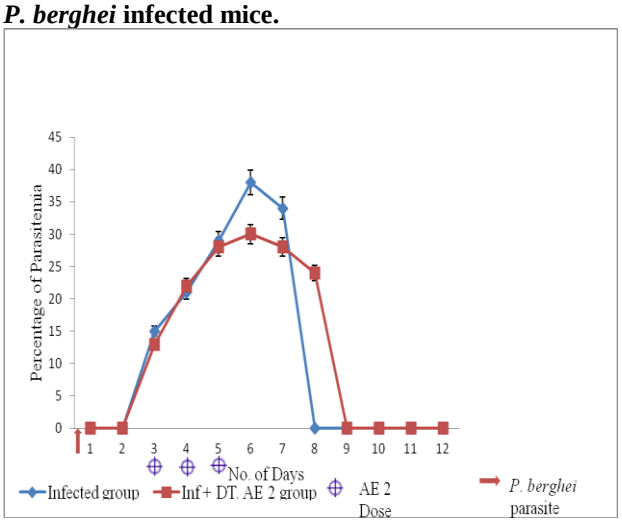

Fig. 2: Effect of therapeutic dose of arteether to *P. berghei* infected mice.

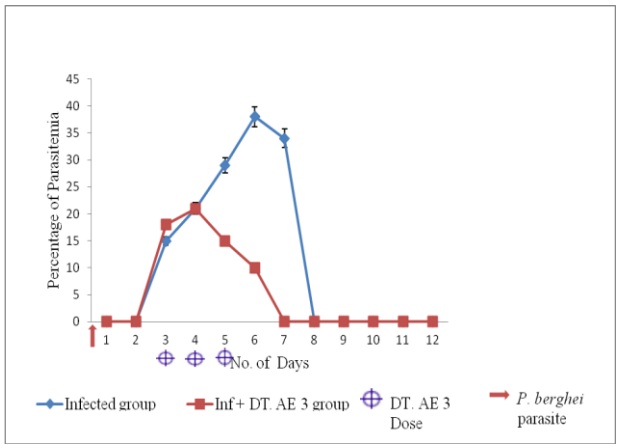

Fig. 3: Effect of double-therapeutic dose of arteether to *P. berghei* infected mice.

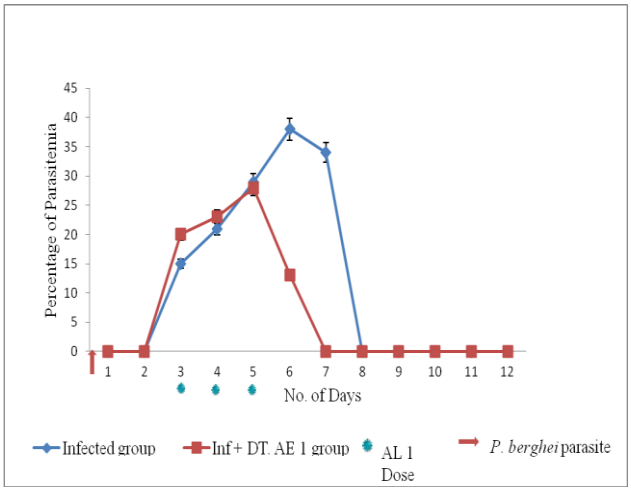

Fig. 4: Effect of sub-therapeutic dose of artemether-lumefantrine *P. berghei* infected experimental mice.

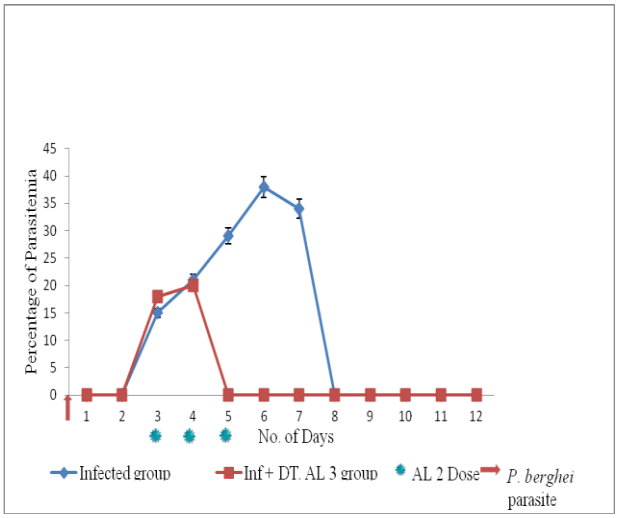

Fig. 5: Effect of therapeutic dose of artemether-lumefantrine to *P. berghei* infected experimental mice.

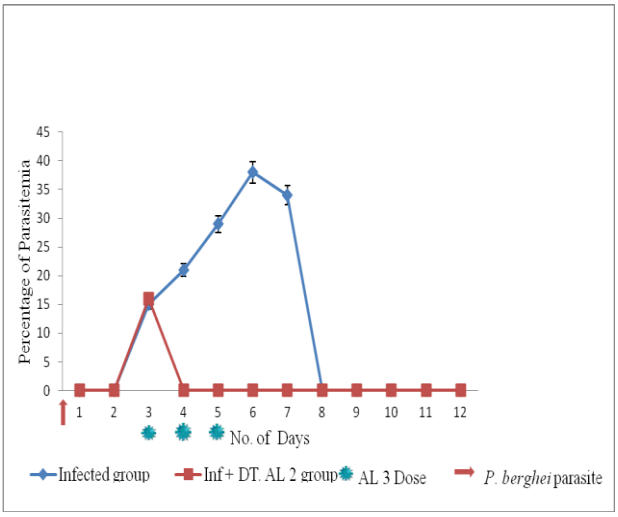

Fig. 6: Effect of double-therapeutic dose of artemether-lumefantrine to *P. berghei* infected experimental mice.

DISCUSSION

In the present investigation, the course of infection of *P. berghei* in infected mice reached peak level of infection (38%) on day 6 which is in correlation with the previous studies. Albino mice showed detectable parasitemia on day 4 post infection of *P. berghei* with about 30% of parasitemia before death of animal and was recorded.[11] Animals infected with 1.0 x 10^6 reached highest parasite density on day 6 with 28.1% mean parasitemia. This later reduced to 17.0% on day 8 and 16.8% on day 9 and death occurring on day 10.[12]

In our experiment, PCT in sub-therapeutic dose of arteether was 240 h (10 days) with peak level of parasite biomass on day 8 with 31%. In therapeutic dose of arteether, parasitemia reached peak level on day 6 with 30% and then parasites disappeared on day 12, PCT was 168 h (7days). PCT for double-therapeutic dose of arteether was 120 h (5 days) with peak parasitemia on day 4 with 21%. Our results are in contrast to the earlier reports where in phase II clinical studies of arteether, it was observed that from the peripheral blood the parasites were disappeared in 80% of the patients at 48 h and in 98% of the patients at 72 h. In phase III multicentric clinical trial with arteether, in uncomplicated malaria PCT was recorded in the range of 24-72 h and in complicated group PCT ranged between 24-120 h.[13] In a randomized controlled trial of artemotil (Beta-arteether) in Zambian children with cerebral malaria demonstrated the mean PCT 53 h for the artemotil treatment group.[14] In a multicentric study, treatment of acute falciparum malaria with beta-arteether the mean PCT was 38.49 h and for alpha/beta-arteether was 36.9 h.[15]

PCT in sub-therapeutic dose of artemether-lumefantrine was 120 h (5 days). Peak parasitemia was observed on day 5 with 28%. Therapeutic dose of artemether-lumefantrine PCT was 72 h (3 days). PCT for double-therapeutic dose of artemether-lumefantrine was 48 h (2 days). Our results are in variance with the earlier reports. In an open label, non-comparative design, children suffering from acute uncomplicated falciparum malaria in Nigeria, were treated with artemether-lumefantrine, a total of 75.3% and 89.9% of patients were free of parasitemia at 24 h and 60 h respectively. While all patients were free of parasitemia by day 3.[16]

In the infected group haemolytic anaemia, leucopenia and thrombocytopenia with low PCV were noticed. These results are in consistence with earlier reports.[17,18,19] After treatment with artemether-lumefantrine the parametric improvement was noticed and parasite burden and PCT was rapid when compared to arteether. Our results are in consistant with the previous reports.

Treatment of uncomplicated malaria in Zambian children with artemether-lumefantrine (AL) on day 3, severe anaemia was observed. After treatment with AL, complete haematological recovery was observed.[20] Haematological abnormalities like decreased levels of red blood cells, haemoglobin and leucocytosis were recorded during *P. berghei* infection in male and female albino mice. The increased level of the haematological parameters were noticed in this study may be due to the antimalarial effect of artemether-lumefantrine on the Plasmodium parasite.[21]

Comparison of artemisinin monotherapy (AE) and artemisinin combination therapy (AL) from the present study can assume that combination therapy has higher efficacy value in clearing parasite biomass and in resolving malaria associated pathological features. Both artemether and lumefantrine has complementary pharmacokinetics and dissimilar modes of action; and hence provide synergistic anti-malarial activity.[22] Artemether is rapidly eliminated from plasma with a half-life of two to three hours, whereas lumefantrine is

eliminated more slowly with a half-life of three to six days and provides a high long-term cure rate after a short treatment course.[22] The combination thus provides rapid clearance of parasitemia and most malaria-related symptoms, coupled with prevention of recrudescence. The fast parasite clearance is due to the rapid absorbance and its main active metabolite dihydroartemisinin (DHA) achieve a fast reduction in parasite biomass and prompt symptomatic improvement while the lumefantrine concentrations that persist in the blood after the three days treatment course eliminate any remaining parasites to prevent recrudescence.[23]

In our experiment we find variation in parasite clearance time and peak level of parasitemia. Both AE and AL showed no recrudescence. The efficacy of study drugs was DT. AL 3 > DT .AL 2 > DT. AE 3 > DT.AE 2 > DT.AL 1 > DT. AE 1. The levels of efficacy are similar to the findings of the recent studies on ACTs in Africa which revealed that AL has higher efficacy rates when compare to other antimalarials.[24,25] In therapeutic dose of both AE and AL the parasite burden is high so also the PCT was high. Moreover, artemisinin resistance is characterized by prolongation in parasite clearance time.[26]

CONCLUSION

Our data suggest that artemether-lumefantrine is a highly efficacious combination with rapid parasite clearance and with low parasite burden. The adverse effects of neither drug were severe enough. But it is evident that Arteether is on the edge of developing resistance after evaluating the above parameters. More studies are needed towards the causes of resistance of antimalarial drugs.

Conflict of Interest

The authors declare they have no conflict of interest.

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