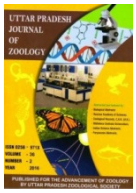
UTTAR PRADESH JOURNAL OF ZOOLOGY

38(4): 124-134, 2018
ISSN: 0256-971X (P)



EFFECT OF ARTEMISININ-BASED COMBINATION THERAPY (ACT) ON HAEMATOLOGICAL PARAMETERS OF EXPERIMENTAL MICE INFECTED WITH *Plasmodium berghei*

M. RAJYALAKSHMI[1], D. MADHU PRASAD[1] AND K. SUNITA[1*]

[1]Department of Zoology and Aquaculture, Acharya Nagarjuna University, Nagarjuna Nagar, 522510, Guntur, Andhra Pradesh, India.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Author MR performed the statistical analysis and wrote the first draft of the manuscript. Author DMP managed the literature searches and author KS designed the study, wrote the protocol and managed the analyses of the study. All authors read and approved the final manuscript.

ARTICLE INFORMATION

Reviewers:
(1) Joshua Raji, Florida International University, USA.
(2) Tabe Franklin Nyenty, University of Ngaoundere, Cameroon.
(3) Greanious Alfred Mavondo, National University of Science and Technology, Zimbabwe.

Received: 03 July 2018
Accepted: 15 October 2018
Published: 23 October 2018

Original Research Article

ABSTRACT

Background: Haematological abnormalities are implicated in malaria and many pharmacological agents used in the treatment of malaria in the tropics. The present study evaluated the effect of different Artemisinin-based combination therapies (ACTs) namely: Artesunate + Amodiaquine (ASAQ), Artesunate + Sulfadoxine Pyrimethamine (ASSP), (Artesunate + Lumefantrine (AL) on the haematological status of *P. berghei* infected mice.

Methods: Course of infection was studied in infected and drug treated experimental mice by observation of blood smears. Haematological parameters were estimated according to the standard protocols.

Results: Haematological parameters like Hb, RBC count, PCV, MCV, MCHC, PLT count were significantly decreased ($P < .05$) and MCH and WBC count were significantly increased ($P < .05$) in *P. berghei* infected group when compared to the control group. Differential Leucocytes Count (DLC) has shown significant increase ($P < .05$) in lymphocyte, neutrophil and monocyte counts but shown significant decrease ($P < .05$) in eosinophil and basophil counts in *P. berghei* infected group when compared to the control group. When these infected mice were subjected to Artemisinin-based combination therapy, the haematological parameters were restored to normal levels.

Conclusion: Among the drug treated groups, ASAQ was found to be more efficacious when compared to the ASSP and AL in restoring the haematological indices and rapid parasite clearance. No recrudescence was

**Corresponding author: Email: drsunitamichael@gmail.com;*

observed in all the treated groups up to 28 day follow-up. Hence, our findings suggest that all the three ACTs are effective and safer but among the three, ASAQ combination therapy is more effective and best haematological recovery was observed when compared to the ASSP and AL combination therapies.

Keywords: *Plasmodium berghei*; parasitaemia; haematological parameters; parasite clearance time; artemisinin-based combination therapy.

1. INTRODUCTION

Malaria is widespread in countries located in tropical and subtropical regions, where an estimated 3.2 billion people, nearly half of the world's population are at risk of infection [1]. Among the five species of *Plasmodium* that infect humans, *Plasmodium falciparum* is the most virulent with the highest rates of complications and mortality as well as the most frequent incidence of red blood cell disorders worldwide [2]. Over 91% of the estimated 445,000 global deaths from malaria in 2016 occurred in Sub-Saharan Africa, primarily among children less than five years of age [3]. The significant decrease in malaria incidence is the result of both preventive measures such as the massive distribution of insecticide-treated nets, vector control strategies, and rapid diagnostic tests as well as the use of artemisinin-based combination therapies (ACTs) in curative therapy. Malaria is typically diagnosed by the microscopic examination of blood using blood films [4].

Some of the symptoms in malaria infections are anaemia, thrombocytopenia, leucocytosis, antioxidant reductions, increased lipid peroxidation and coma. Patients with malaria often develop haematological complications and alterations in biochemical parameters [5]. The manifestations of malaria illness result from the infection of red blood cells by asexual forms of the malaria parasite. Hence, the infection of the red blood cells makes malaria a potential multi-system disease because all organs of the body depend on blood for their metabolism [6,7]. The infection of red blood cells by malaria parasites may lead to structural, biochemical and physiological modifications of the red blood cells, therefore, resulting in some life-threatening symptoms of malaria. Moreover, malaria parasites attack the red blood cells thereby causing their lysis which may result in reduced haemoglobin level and packed cell volume [8].

In healthy conditions, blood cells are adequately produced to a particular range based on the sex and age of the individual. The white blood cells (neutrophils, lymphocytes, eosinophils, monocytes and basophils) are the lines of defense against pathogenic invasion in animals and their percentages in differential count would give some insight on the kinds of infection an individual has. For instance, neutrophilia is found in bacterial infection; neutropenia in other nonbacterial related diseases; lymphocytosis in some viral and parasitic infections: basophils in blood clotting disorders; monocytes, in some bacterial infections [8].

Several patho-physiological factors such as the parasite biomass; malaria toxins and inflammatory response; cytoadherence rosetting and sequestration; altered deformability and fragility of parasitized erythrocytes; endothelial activation dysfunction and injury; and altered thrombostasis have been found to be involved in the development of severe malaria [9].

The species *Plasmodium berghei* is often used in predicting the treatment outcome of any suspected antimalarial agent due to its high sensitivity to chloroquine, thereby making it an appropriate parasite for this study [10]. *Plasmodium berghei* have been used in studying the activity of potential antimalarials in mice [11] because it produces diseases similar to those of human plasmodium infection [12].

Since 1994, artemisinins have been used in ACTs to treat uncomplicated malaria. ACTs combine 2 active ingredients, artemisinins and another antimalarial drug with different mechanisms of action. It has been reasoned that in ACTs, the partner drugs are chosen on the basis of their pharmacokinetic properties, which include much longer plasma half-lives (days to weeks) than those of artemisinins (1 to 2 h). While artemisinins are eliminated very rapidly from the body, the remaining parasites are exposed to the associated long-acting drug well after the end of the usual 3-day ACT course [1] which may mean the presence of these other drugs may predispose to post treatment adverse effects on the rheological parameters of blood. Thus, ACTs are the most effective antimalarial medicines available today, and they have replaced quinolines and antifolates as the first-line treatment for uncomplicated *P. falciparum* malaria in most endemic countries.

Five ACTs are currently used, namely, artemether+lumefantrine (AL), artesunate+

amodiaquine (ASAQ), artesunate+mefloquine (ASMQ), artesunate+sulfadoxine pyrimethamine (ASSP) and dihydroartemisinin+piperaquine (DHA+PPQ) [1]. A sixth ACT, artesunate+pyronaridine (MMV-supported projects: Medicines for Malaria Venture) [13] was recently approved, and unfortunately, its current efficacy on day 42 was below 90% in Western Cambodia, an artemisinin-resistance area [14]. In 2016, 409 million ACT-based treatments were applied worldwide [1].

The recommended treatment for malaria is a combination of antimalarial medications that includes an artemisinin. Artemisinin and its semi-synthetic derivatives are a group of drugs that possess the most rapid action of all current drugs against *P. falciparum* malaria. Artemisinins can be used alone, but this leads to a high rate of recrudescence (return of parasites) and other drugs are required to clear the body of all parasites and prevent recurrence. The WHO has recommended that artemisinin combination therapies (ACT) should be the first-line therapy for *P. falciparum* malaria worldwide [3]. Thus, the present study was aimed at investigating the impact of ACTs on some haematological indices of *P. berghei* infected experimental mice.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Thirty male Swiss albino mice each weighing 25-30 g were divided in to 5 experimental groups each with 6 animals (n = 6). Animals were allowed to acclimatize for one week before initiation of the experiment. They were housed in plastic cages with rice husk as beddings, provided with access to commercial pellet food and 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 [15].

2.2 Parasite

Chloroquine sensitive *P. berghei* ANKA strain parasites were maintained by intraperitoneal inoculation of 1×10^7 infected erythrocytes to naïve mice. A standard inoculum consisting of 1×10^7 parasitized erythrocytes was prepared from the infected donor mice with >25% parasitaemia, and used to infect experimental mice.

2.3 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 1×10^7 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 [16]. The day of inoculation was defined as Day 0 and subsequent days as Day 1, Day 2, and Day 3 up to Day 28.

2.4 Drugs and Dosage Regimens

In the present work, three Artemisinin-based combination drugs were used namely Atesunate + Amodiaquine (AS+AQ), Artesunate + Sulphadoxine Pyrimethamine (AS+SP), Artemether + Lumefantrine (AL). All the drug dosages were given according to the body weight of mouse by following standards of World Health Organization (WHO).

2.4.1 Artesunate + Amodiaquine (ASAQ)

The combination drugs of Artesunate (50 mg) tablet and Amodiaquine Hydrochloride (153.1 mg) tablet from IPCA Laboratories Limited, Mumbai, India were used in the present experiment as the first ACT. Artesunate (50 mg) tablet was dissolved in 50 ml of distilled water to obtain the stock solution concentration of 1 mg/ml. And 153.1 mg tablet of Amodiaquine dissolved in 150 ml of distilled water to obtain the stock solution concentration of 1.02 mg/ml. The WHO dosage regimen is Artesunate 4 mg/kg + Amodiaquine 10 mg/kg once a day for 3 days. So in the present experiment, the same WHO recommended dosage regimen was followed and administered to the infected mice for 3 days by oral gavage according to the body weight.

2.4.2 Artesunate + Sulphadoxine Pyrimethamine (ASSP)

The combination drugs of Artesunate (200 mg) tablet and Pyrimethamine (25 mg) + Suphadoxine (500 mg) tablet (LARINATE-200 kit) from IPCA Laboratories Limited, Mumbai, India was used in the present experiment as the second ACT. Artesunate stock solution (1 mg/ml) was prepared as was in 2.4.1. And Pyrimethamine (25 mg) + Sulphadoxine (500 mg) tablet was dissolved in 100 ml of distilled water to obtain the stock solution concentration of 5.25 mg/ml. The WHO dosage regimen is Artesunate 4 mg/kg once daily for 3 days and Sulphadoxine + Pyrimethamine as single dose of 25 mg/kg + 1.25 mg/kg on Day 1, which was administered orally. The above WHO dosage regimen was followed in the present experiment.

2.4.3 Artemether + Lumefantrine (AL)

The third combination drug used was Artemether (20 mg) and Lumefantrine (120 mg) tablet (LUMERAX-20 DT) from IPCA Laboratories Limited, Mumbai, India. The tablet Artesunate (20 mg) and Lumefantrine (120 mg) was dissolved in 50 ml of distilled water to obtain the stock solution concentration of 2.8 mg/ml respectively. The WHO dosage regimen is Artemether 1.5 mg/kg and Lumefantrine 9 mg/kg at 0, 8, 24, 36, 48 and 60 hour. The same WHO regimen was followed and 6 doses were given on 3 consecutive days.

A combination therapy would be helpful in simultaneous use of two or more blood schizontocidal drugs with independent modes of action and different biochemical targets in the parasite [17].

2.5 Animal Groups

The mice were divided into following 5 groups with 6 mice (n = 6) in each group:

Group 1 (Control): The mice were given only distilled water.

Group 2 (Infected Non-treated): The mice were infected with *P. berghei* antigen.

Group 3 (Infected + ASAQ): The mice were first infected with *P. berghei* antigen and then treated with Artesunate + Amodiaquine combination.

Group 4 (Infected + ASSP): The mice were first infected with *P. berghei* antigen and then treated with Artesunate + Sulphadoxine Pyrimethamine combination.

Group 5 (Infected + AL): The mice initially were parasitized with *P. berghei* and then treated with Artemether + Lumefantrine combination.

In all the experimental groups (both infected non-treated and infected- treated mice), parasitaemia was estimated throughout the experimental period daily by observation of Giemsa stained blood smears under the microscope. On 7th day of the of experiment period, the required number of mice were euthanized with chloroform and the blood samples were collected through cardiac puncture in sterile labeled EDTA coated tubes for haematological investigation.

2.6 Estimation of Course of Infection in *Plasmodium berghei* Infected Mice

Thin blood films were prepared on clean slides, initially fixed with methanol. A large drop of blood is put at the center of a clean dry slide. The drop is spread with an applicator slide, and then the smear is thoroughly dried in a horizontal position. Blood smears were stained with Giemsa stain 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 Red Blood Corpuscles (RBCs) were in an evenly distributed monolayer followed by the x100 oil immersion objective. A minimum of 1000 RBCs were counted and among those, number of infected RBCs will be recorded. The percent of infected RBCs (parasitaemia) was determined by enumerating the number of infected RBCs in relation to the number of uninfected RBCs [18] as follows.

$$\text{Percentage of Parasitaemia} = \frac{\text{No. of infected RBCs}}{\text{No. of RBCs counted}} \times 100$$

2.7 Estimation of Haematological Parameters

In the present investigation, the haematological changes in all the experimental groups were evaluated. Whole blood was drawn to estimate the haematological abnormalities especially anaemia, thrombocytopenia and reduced blood counts in malaria. Haemoglobin (Hb) was estimated by Sahli's haemoglobinometric method [19], red blood cell (RBC) count, white blood cell (WBC) count and platelet count (PLT) were estimated by using improved Neubauer chamber, differential leucocyte count (DLC) was estimated by examination of blood smear [20, 21] packed cell volume (PCV) was calculated by micro haematocrit method, mean cell haemoglobin (MCH) was calculated as Hb/RBC × 10, mean cell haemoglobin concentration (MCHC) was calculated as Hb/PCV × 100 and mean cell volume (MCV) was calculated as PCV/ RBC × 10.

2.8 Statistical Analysis

Results of individual parameters were expressed as mean ± standard deviation. The comparison between the experimental groups was performed by Student t-test using MINITAB 11.12.32 Bit statistical package and graphs were drawn in MS Excel. The results were statistically significant at $P < .05$.

3. RESULTS

3.1 Physical Behavioural Changes In *P. berghei* Infected Non-treated Group

In *P. berghei* Infected non -treated group the clinical manifestation of sluggishness, inactivity and coming together at the corners of the cages were observed when compared to the healthy mice, may be due to

the hyperparasitaemia. This type of behavior was observed between 4- 7 days of *P. berghei* infection.

3.2 Course of Infection to *P. berghei* in Experimental Mice

3.2.1 *P. berghei* infected non-treated group

During the study of course of infection, *P. berghei* parasite was given to the experimental mice on Day 0. After inoculation the parasitaemia was first appeared on Day 3 (72 hours). Then the parasitaemia was gradually increased up to the peak level on Day 7. On Day 3, initial parasitaemia was 19%, on Day 4 with 23%, on Day 5 with 27%, on Day 6 with 32% and on Day 7 with 36% of parasitaemia. High rate of parasitaemia was observed on 7th day post inoculation after which all the mice died due to heavy infection by Day 8 (Fig. 1).

3.2.2 *P. berghei* infected + ASAQ treated

In this group, initial parasitaemia was 20% on Day 3. On Day 3, Day 4 and Day 5; the therapeutic dose of ASAQ combination drug was administered orally. Then the parasitaemia was decreased to 8% on Day 4. On Day 5, the parasitaemia was 0% and so the parasite clearance occurred within 48 hours. No recrudescence was observed during the follow-up of 28 days. Hence, the survival rate was 100% and parasite clearance time (PCT) in ASAQ treated mice was 2 days (48 hours) (Fig. 1).

3.2.3 *P. berghei* infected + ASSP treated

In this group, the initial parasitaemia was 21% on Day 3. On Day 3, Day 4 and Day 5; the therapeutic dose of ASSP drug was administered orally. Then the parasitaemia decreased to 10% on Day 4. On Day 5, the parasitaemia was 2% and on Day 6 with 0%. No recrudescence was observed during follow-up of 28 days. Hence, the survival rate was 100% and parasite clearance time of (PCT) in ASSP treated mice was 3 days (72 hours) (Fig. 1).

3.2.4 *P. berghei* infected + AL treated

In this group, the initial parasitaemia was 19% on Day 3. Then the mice were treated with AL combination drug for 3 consecutive days orally on Day 3, Day 4 and Day 5. On Day 4 the parasitaemia was 11%, on Day 5 parasitaemia decreased to 3% and on Day 6 no parasitaemia was observed. Also no recrudescence was observed during the follow-up of 28 days. Hence, the survival rate was 100% and parasite clearance time (PCT) in AL treated mice was 3 days (72 hours) (Fig. 1).

3.3 Changes in Haematological Parameters in Different Experimental Mice

In the present study, the haematological changes were evaluated in control, *P. berghei* infected and three drug treated groups i.e., ASAQ, ASSP and AL treated mice.

The Table 1, shows the comparison of haematological values among the *P. berghei* infected non-treated and treated groups. Decrease in Hb, RBC, PCV, MCV, MCHC and PLT count was observed in infected group. And increase in MCH, WBC count, % of neutrophil, % of lymphocyte, and % of monocyte was observed infected group.

But in treated groups, these parameters were restored to normal levels significantly ($P < .05$). And best recovery of these parameters was noted in the Artesunate + Amodiaquine (ASAQ) treated group than ASSP and AL treated groups (Table 1).

4. DISCUSSION

The haematological and biochemical indices have been reported to be reliable parameters for assessment of health status of animals [22, 23]. Evaluating the complete blood count in order to ascertain changes in erythrocytes, leucocytes and platelets guide the diagnosis of anaemia, leucocytosis and thrombocytopenia in malaria infections.

Various treatment regimens has been developed to treat malaria and in recent times multi-drug therapies have been adopted as the most effected means of combating malaria. Artemisinin-based Combination Therapy (ACT) remains the preferred first line and safe treatment of falciparum malaria because of its rapid clearance of parasitaemia, and reduction of changes of deaths in cases of severe malaria [24]. Thus in the present study, we examined the effect of Artemisinin-based combination therapeutic drugs namely: ASAQ, ASSP and AL on haematological indices of *P. berghei* infected mice.

In the experimental mice; the clinical manifestations of sluggishness, inactivity and coming together at the corners of the cages by the *P. berghei* infected mice when compared to the control group, may be due to hyperparasitaemia. This is in consonant with the report of Centres for Disease Control and Prevention [25] on the clinical manifestations of malaria.

In the present investigation, the course of infection in *P. berghei* infected non treated group mice reached peak level of infection (i.e., 36%) on Day 7 after which all the mice died due to heavy infection which

is in correlation with the previous studies. Rajan et al. [26] reported that albino mice shown peak level of parasitaemia 49.5%±3.53 on Day 7 and Badejo et al. [27] reported the peak parasitaemia of 38% on Day 9 during *P. berghei* infection in Swiss albino mice and all the mice were died by Day 10. Thus the present results confirm the previous results where the mice died after the parasitaemia reached to peak level.

In our investigation Hb, RBC count, PCV, MCV, MCHC were significantly decreased ($P < .05$) in infected mice when compared to control mice. Anaemia is observed to be one of the basic pathological symptoms in *P. berghei* infected mice [28]. And high rate of parasitaemia caused more destruction of red blood cells which results from a reduction in erythrocyte count or reduction in the concentration of haemoglobin in each erythrocyte [29]. The reduction in RBC and Hb levels is noticed as an indication of haemolytic anaemia. This may be due to haemolysis of parasitized red blood cells and accelerated removal of parasitized red blood cells [30]. While in blood stage propagation of *P. berghei* ANKA, PCV was markedly decreased due to haemolysis of erythrocytes and insufficient mature erythrocyte production [31] which correlates with the present finding.

Our results also correlate with the previous reports of Momoh et al. [32], Itemobong et al. [33] and Obeagu et al. [34] where MCH has significantly increased and MCHC has significantly decreased in infected mice when compared to control mice. These infected mice when treated with ASAQ, ASSP and AL treated groups, all the haemotological indices recovered to nearly normal values.

The Hb and other haemotological parameters were slightly more recovered in ASAQ treated group when compared to ASSP and AL treated groups. This finding confirms the earlier report that artemisinin drugs cause less antimalarial drug-related falls in packed cells volume during treatment [35]. Koram et al. [36] reported that artemisinin-based combinations (ACTs) are known to effect rapid fever and parasite clearance and the wide-spread use of more effective antimalarial drugs would probably result in significant clinical and haematological benefits.

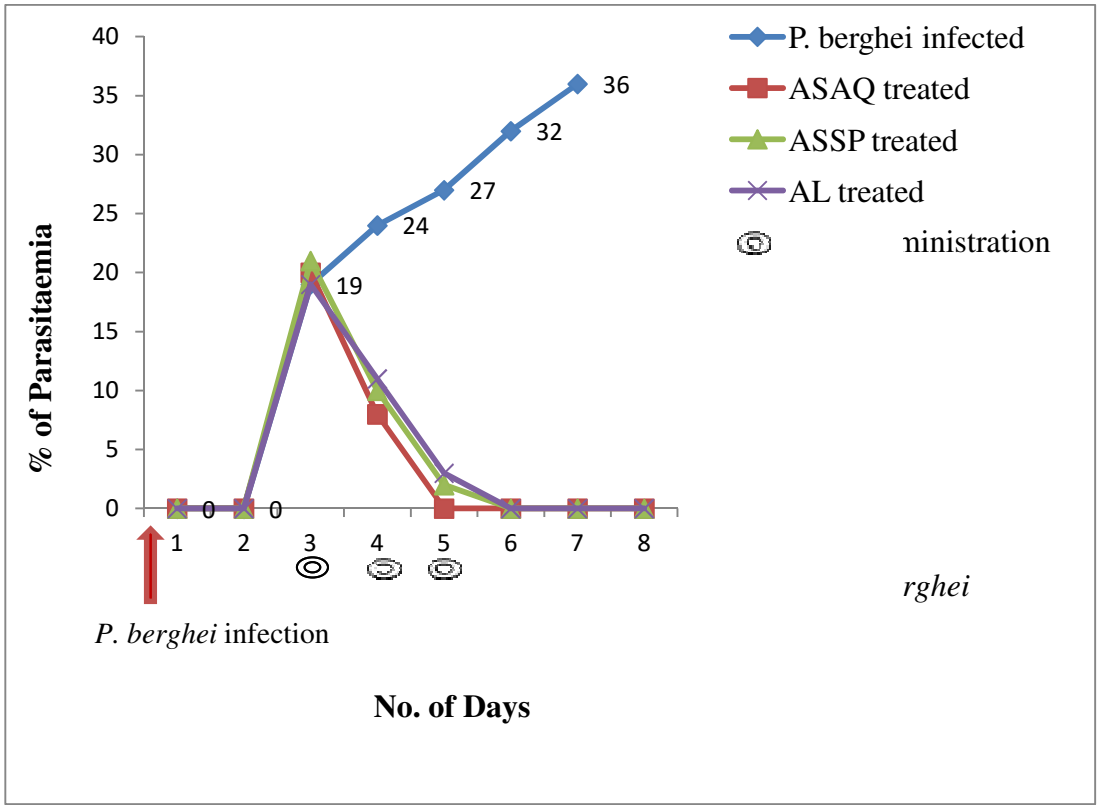


Fig. 1. Course of infection to *Plasmodium berghei* in infected non-treated and teated mice during Artemisinin-based combination therapy

Table 1. Changes in haematological indices in *Plasmodium berghei* infected non-treated and treated mice with Artemisinin-based combination therapy

S No.	Parameter	Control Non-infected (Normal) (n=6)	*P. berghei* Infected non-treated mice (n = 6)	*P. berghei* Infected + ASAQ treated (n = 6)	*P. berghei* Infected + ASSP treated (n = 6)	*P. berghei* Infected + AL treated (n = 6)
1	Hb (g/dL)	14.6±0.00816	10±0.0816	14.4 ±0.163 P = 0.0000* t = 47.10	14.1 ±0.163 P = 0.0000* t = 44.91	13.95 ±0.129 P= 0.0000* t = 51.72
2	Total RBC (10^6/mm^3)	9.4±0.275	7.2±0.163	9.27 ±0.275 P = 0.0001* t = 9.84	9.01 ±0.095 P = 0.0000* t = 13.89	8.97 ±0.250 P = 0.0001* t = 8.54
3	PCV (%)	40±0.816	21.8±0.849	36 ±0.126 P = 0.0000* t = 28.81	34.9 ±0.650 P = 0.0000* t = 24.56	34.0 ±0.074 P = 0.0000* t = 24.90
4	MCV (fL/cell)	53.2±0.197	48.05±0.173	52.1 ±0.021 P = 0.0000* t = 46.52	52 ±0.017 P = 0.0000* t = 45.42	52.01 ±0.021 P = 0.0000* t = 45.37
5	MCH (pg/cell)	16.0±0.141	19.0±0.216	15.8 ±0.129 P = 0.0000* t = 25.03	15.76 ±0.160 P = 0.0000* t = 24.031	15.61 ±0.062 P = 0.0000* t = 30.11
6	MCHC (g/dL)	28.1±0.294	26.9±0.129	29.2 ±0.163 P = 0.0000* t = 21.62	27.4 ±0.365 P = 0.05* t = 2.32	27.7 ±0.770 P = 0.05* t = 2.43
7	PLT count (10^3 /mm^3)	1027±1.83	685±0.816	1000 ±1.71 P = 0.0000* t = 382.02	998 ±0.645 P = 0.0000* t = 601.92	995 ±1.83 P = 0.0000* t = 310
8	WBC (cells/mm^3)	5.9±0.126	6.9±0.171	5.75 ±0.129 P = 0.0000* t = 10.98	5.65 ±0.129 P = 0.0000* t = 11.91	5.60 ±0.183 P = 0.0000* t = 10.60

9	Lymphocytes (%)	68±0.170	80±0.081	67 ±0.126 P = 0.0000* t = 172.33	67.4 ±0.0171 P = 0.0000* t = 302.04	67.01 ±0.014 P = 0.0000* t = 313.52
10	Neutrophils (%)	17.01±0.038	36.05±0.603	16.22 ±0.171 P = 0.0000* t = 63.29	15.92 ±0.126 P = 0.0000* t = 65.37	16.02 ±0.330 P = 0.0000* t = 58.26
11	Monocytes (%)	1.8±0.017	1.9±0.009	1.78 ±0.009 P = 0.0000* t = 18.46	1.75 ±0.0129 P=0.0000* t = 18.98	1.76 ±0.002 P = 0.0000* t = 12.44
12	Eosinophils (%)	1.11±0.018	1.05±0.008	1.10 ±0.020 P = 0.0027* t = 4.92	1.09 ±0.009 P = 0.0003* t = 7.55	1.07 ±0.008 P = 0.013* t = 3.46
13	Basophils (%)	0.12±0.0171	0.06±0.012	0.09 ±0.017 P = 0.042* t = 2.57	0.08 ±0.081 P = 0.097^{NS} t = 1.96	0.04 ±0.011 P = 0.028* t = 2.89

*Data are expressed as Mean ± Standard Deviation, *Significant difference at P < 0.05, t > 2.306 compared with infected group. NS - Not significant.*

Malaria is a complex multi-system disorder involving several inflammatory mediators. Monocyte, macrophages, endothelial cells, neutrophils and PLTs all express pattern recognition receptors, primarily toll-like receptors [37]. In this study total platelet count (PLT) was significantly decreased in infected mice when compared to control mice. Thrombocytopenia is one of the haematological hallmarks of acute malaria. Despite the occurrence of severe bleeding in severe malaria or co-infection [38], malaria related thrombocytopenia is usually observed at frequencies ranging from 24-94% [39]. A reduction in the red blood cells and platelet counts (pancytopaenia) recorded in this investigation confirms earlier reports [30,33,40].

We also observed the total white blood cells (WBC) counts in experimental mice. WBC count was significantly increased (leucocytosis) in infected mice. This finding was also observed by Momoh et al. [41].

The increase of neutrophils, lymphocytes and monocytes in infected mice in the present study is in consistent with the previous reports of Itemobong et al. [33]. The activation of neutrophils in the initial stages of malaria infection is well supported by Graca-Souza et al. [42] and Mohammed et al. [43]. Monocytes were also significantly increased in the infected group. This leucocytosis including lymphocytosis and monocytosis was in accordance with observations in children suffering from *falciparum* malaria for which high level of lymphocytes was reported [44].

The basophil and eosinophil counts decreased significantly in infected mice during our study. The leucocytosis and eosinopenia in this study shows a correlation with another previous study which showed that *P. falciparum* and *P. vivax* infected patients from Phobphra Hospital, Tak Province, Thailand lead to increase in the absolute neutrophil count and the decrease in the absolute eosinophil count [45].

The results from this study have shown the better haematological recovery in the ASAQ treated group when compared to ASSP and AL treated groups which are in correlation with the previous reports [46]. And the parasite clearance time (PCT) was 48 hours in ASAQ treated group but in ASSP and AL treated groups the PCT was 72 hours. Finally, the present investigation reveals that ASAQ combination is more effective than other artemisinin-based combination drugs of the study.

5. CONCLUSION

Our study stated that all three combinations ASAQ, ASSP and AL were efficacious and safety with rapid parasite clearance. Also shown good haematological recovery in the drug treated groups. But ASAQ treated group has shown slightly more recovery of haematological indices when compared to other treated groups. Finally, we conclude that ASAQ, ASSP and AL combination drugs are effective for malaria treatment but ASAQ was found to be the more efficacious combination.

ETHICAL APPROVAL

Animal experiments were designated and approved with Ref. No. ANUCPS/IAEC/AH/Protocol/2/2014 by Institutional Animal Ethics Committee (IAEC) of ANU College of Pharmacy, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India.

ACKNOWLEDGEMENT

The authors are thankful to the Co-ordinator, Department of Zoology and Aquaculture, Acharya Nagarjuna University, Guntur, Andhra Pradesh for providing necessary facilities through UGC-SAP-DRS – Phase III and UGC-FIST – Phase I funding. The first author is highly thankful to UGC, New Delhi for providing RGNF to carry out the present research work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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