EFFECT OF CO-ADMINISTRATION OF VITAMIN A AND ARTESUNATE IN MALARIA-INFECTED ALBINO MICE

ABSTRACT

Malaria is a potentially fatal illness that results from infection by parasitic protozoa, which spread to humans via the bites of mosquitoes that have already been infected with the parasite. This research examines the impact of the simultaneous administration of Vitamin A and Artesunate on Plasmodium berghei-infected albino mice. A cohort of sixteen (16) albino mice, with a mean weight of 28g and indeterminate gender, were subjected to p.berghei inoculation. The mice were separated into four groups, each consisting of four animals. Artesunate was administered to Group A, distilled water was administered to Group B, Group C received antioxidant treatment in the form of vitamin A, and Group D was subjected to a combination treatment of Artesunate and Vitamin A. The study monitored the parasitemia level, hematocrit (PVC), percentage mortality rate, and change in body weight following the administration of various treatments, including an antimalarial drug (Artesunate), distilled water, Vitamin A, and a combination therapy of both Artesunate and Vitamin A. The co-administration of vitamin A and Artesunate may decrease the effectiveness of Artesunate in treating malaria infection. The research illustrates the intricate relationship between Artesunate and antioxidant (vitamin A) in mice infected with malaria. The efficacy of Artesunate in clearing the parasite was demonstrated by the reduction of Parasitaemia levels from +2(++) on the 5th day to +3 (+++) on the 7th day, followed by a decrease to +2(++) on the 9th day, and ultimately to +1(+) Parasitaemia on the 10th day. The co-administration of Vitamin A and Artesunate resulted in a reduction of the latter's efficacy in treating malaria, particularly in cases where the parasitemia level was at +3(+++) on the 5th, seventh, and 11th days and +2(++) on the ninth day. This procedure suggests that Vitamin A acted as an antagonist to the antimalarial effects of Artesunate.

Furthermore, the mortality rate was 50%, and the PCV was reduced compared to Group A. Artesunate, a pro-oxidant that generates free oxygen radicals that act against the parasite. However, the presence of antioxidants like Vitamin A potentially counteracted the effects of pro-oxidants, indicating some form of pharmacological antagonism. The research findings indicate that the co-administration of vitamin A and Artesunate may reduce therapeutic effectiveness. Clinical interventions are recommended to be implemented to regulate the simultaneous administration of Artesunate and Vitamin A, as this may impact the therapeutic objective of malaria treatment.

Keywords: Plasmodium berghei, Parasitaemia, Artesunate, Vitamin A, Mice.

INTRODUCTION

Despite the significant efforts invested in controlling Plasmodium spp. in endemic countries, malaria remains a disease of public health significance, as per the World Health Organization's report in 2014. The onset of the COVID-19 pandemic in early 2020 has presented a complex challenge to the fight against infectious diseases in numerous African nations, where public health services were already grappling with significant obstacles (Monroe *et al.*, 2022). Significant advancements have been made on a global scale in the reduction of malaria cases in Africa. The epidemiology of malaria infection has persisted or even escalated in some areas of the African continent, necessitating immediate inquiry and measures to reinforce its management, as per the World Malaria Report of 2021. According to a recent malaria report by the World Health Organization (WHO), estimated 240 million cases of malaria and 620,000 deaths were attributed to the disease globally in 2021 (Monroe *et al.*, 2022). The present dataset indicates a rise of approximately 14 million instances and 69,000 fatalities compared to the previous year, 2020. According to Monroe *et al.*, (2022), the COVID-19 pandemic has impaired health systems, which may have contributed to a 10-case increase in incidence per population of 1000 at risk in 2020.

The etiology of severe malaria in humans is predominantly attributed to Plasmodium Falciparum. In contrast, other species of Plasmodium, such as P. vivax, P. ovale, and P. malaria, are also known to cause the disease. The presence of human Plasmodium among domestic animals may account for malaria's notable prevalence and persistence in certain endemic regions, as reservoir hosts can exhibit varying presentation patterns (Munirah *et al.*, 2023). The successful acclimatization of Plasmodium berghei, a malaria-causing parasite, to various rodent hosts has been established as a reliable model for studying the disease (Joachim M.M., Taco W.k., 2015).

Of the various Plasmodium species, P. falciparum is distinguished by its ability to infiltrate all red blood cells and induce multiple infections within a single cell. This characteristic leads to rapid proliferation and

exacerbation of the illness. Red blood cells undergo structural and functional alterations, including sequestration, inflammation, and endothelial dysfunction, which are associated with the development of severe malaria. The study was conducted by Gao and colleagues in 2019.

The World Health Organization has suggested the utilization of artemisinin-based combination therapy as a means to impede or postpone the emergence of resistance. Therefore, the acquisition of novel medications necessitates the utilization of Artemisinin as the most extensively employed framework for the synthesis of artificial compounds. The present study provides an overview of artemisinin-based drugs and their derivatives, encompassing hybrid derivatives and dimers, trimers, and tetramers containing an endoperoxide bridge (Luiz *et al.*, 2018). These drugs have demonstrated efficacy against P. falciparum strains that have developed resistance to other antimalarial agents. However, they are ineffective in treating malaria caused by P. vivax, P. ovale, and P. malaria. The cleavage of the endoperoxide bond at the infected cell, which generates radical intermediates and leads to subsequent chemical rearrangements, is a crucial factor in the antimalarial activities of Artemisinin, as noted by Shikha *et al.*, in their recent study (2022).

In recent decades, research studies have demonstrated the efficacy of Artesunate against malaria as well as its potential anticancer effects on different tumor cell lines, both in vitro and in vivo, as reported by Zhang D. *et al.*, in 2017. Furthermore, Artesunate is significant in treating lupus, as indicated by Ismail M., Du Y., Ling L., and Li X. in 2019. Additionally, Shenoy R.K. *et al.*, in 2019 and Kumaran U. *et al.*, in 2021 have reported the efficacy of Artesunate against several viruses. Moreover, Artesunate exhibits anti-diabetic properties, as Li Z. *et al.*, reported in 2019.

The anti-tumor properties of Artesunate have been observed to induce non-apoptotic cell death through various mechanisms, including autophagy, oncosis, and ferroptosis. Several studies have reported these

findings (Zhu W. *et al.*, 2019; Ooko E. *et al.*, 2015; Shi X. *et al.*, 2017; Jiang F. *et al.*, 2018). The compound, known as Artesunate, has been observed to impact various critical processes in cancer development. This includes inhibiting cancer cell proliferation and invasion, induction of cell cycle arrest, disruption of cancer signaling pathways, induction of oxidative damage, and stimulation of cell apoptosis. The compound in question exerts its effects through the inhibition of angiogenesis and as a metastasis agent, as reported by Xu *et al*., in 2020 and Zhao *et al.*, in 2013.

Retinyl esters are the primary dietary source of vitamin A, a crucial micro-essential nutrient. Furthermore, β-carotene, a pro-vitamin A, is a precursor to vitamin A in green and yellow vegetables. Upon ingestion, it converts to retinol within the body (Noriko *et al.*, 2022).

The essentiality of Vitamin A lies in its role in promoting skeletal growth, facilitating vision, enabling cell differentiation, and supporting reproduction. In comparison, some research studies have indicated the potential benefits of Vitamin A in disease prevention. The co-administration of Artemisinin with certain nutritional supplements, particularly Vitamin A, may impact its efficacy in treating malaria infection.

MATERIALS AND METHODS

MATERIALS:

Acquisition of parasite: The parasite was obtained from the Research Laboratory of the College of Medicine, University of Lagos, and was authenticated by a microbiologist in Nigeria.

Acquisition of drugs: The drugs used (Artesunate and Vitamin A) were obtained and authenticated by a University chemist in Nigeria.

Preparation of drugs: The powered drug of Artesunate was diluted with distilled water to a level of 160mg/80mls which was kept as the stock. One millimeter (1ml) of the stock was later diluted with 9mls of distilled water making the concentration 0.2mg/ml.

Parasite Inoculation: The mice were infected with parasites from parasitized blood by cardiac puncture of an infected mouse. 0.2ml of infected blood was diluted in 9.8mls of normal saline. The mice were inoculated on day 1 intraperitoneally with 0.2ml parasitized saline suspension. Development of Parasitemia was monitored by microscopic examination until day 5 when it was established.

Experimental Animals: Sixteen (16) mice were housed in the Animal House of the College of Medicine, Ambrose Ali University Ekpoma, Edo State, and allowed to acclimatize for two weeks prior to the commencement of the experiments. All the animals were given food and water ad libitum. The mice were divided into 4 treated groups of 4 mice per group, the mice were also inoculated from day 1 intraperitoneally with 0.2ml parasitized saline suspension. Development of Parasitemia was monitored by microscopic examination until day 5 when it was established. The experimental mice were weighed at the beginning and at the end of the study.

METHODOLOGY

EXPERIMENTAL DESIGN:

A total number of 16 mice (male and female) were distributed randomly into **four** groups (four mice/group). The experiment lasted for a period of ten days during which the inoculated parasitized mice were grouped as follows:

Group A (Artesunate control group) received Artesunate at 0.7mls/kg for day 6 and 0.35mls from day 7 to day 10.

Group B (The negative control group) received 0.5ml/kg of distilled water daily from day 6 to day 10.

Group C (The positive control group) received 0.03ml/kg of Vitamin A daily from day 6 to day 10.

Group D (Artesunate and Vitamin A control group) received 0.03ml/kg of Vitamin A and Artesunate at 0.35mls/kg from day 6 to day 10.

ANIMAS SACRIFICE:

At the end of the experiment (day 11), all the animals were humanely sacrificed. Blood was collected through the tail vein puncture of the animals in a heparinized centrifuge tube under deep anesthesia with chloroform. The blood collected was centrifuged using a centrifuge machine at 10,000 rmp for five minutes and the serum collected was subjected to packed Cell volume analysis and the assessment of Malaria Parasite count.

ASSESSMENT OF MALARIA PARASITE COUNT:

A semi-quantitative parasite count was done through the tail vein puncture, a drop of blood was collected on a clean slide to get a thick film and allowed to dry and a drop of gemisa stain which was diluted with distilled water at a ratio of 1:10 was dropped on the smear. The smear was left for 5 minutes which enabled the blood cells and the malaria parasite to be viewable under the microscope and later flooded

with distilled water and allowed to dry, a drop of emulsion oil was dropped on the smear and the smear was viewed under the microscope and parasite seen was recorded.

PACKED CELL VOLUME:

This procedure was done by collecting blood from the tail vein into heparinized microhematocrit tubes to 1cm from one end of the tube and the other end of the tube was sealed with a flame. The preparation is centrifuged in a timed micro hematocrit centrifuge for 5 minutes. With a special microhematocrit reader employed for this purpose, the ratio of the Packed Cell to the volume of blood in the heparinized tube can be read and converted to the percentage of the Packed Cell to the whole blood.

RESULTS

PARASITE LEVEL:

During the period of post-inoculation which is days 5,7,9 and 11, the clearance level of Parasitaemia was observed and from Table 1, **Group A** had a clearance level of +2, and its clearance increased to +3 on day 7 and then went ahead to decrease to +2 and subsequently to +1 and this could be due to the administration of Artesunate.

Group B clearance level for days 5 and 7 was +3 and on days 9 and 11, it increases to +4 which could be attributed to the growth of parasitemia when compared to Group A, C, and D without any drugs inhibiting its growth.

Group C clearance level on day 5 was +2 and for days 7, 9, and 11, it increases to +3 which could be that vitamin A no effect in antagonizing the parasitemia present but rather, the parasitemia continues its growth but at a slower rate when compared to Group B.

Group D clearance level on days 5 and 7 was +3 which was normal without an increase or decrease but on day 9 it was +2 which was a slight decrease and it could be attributed to the presence of artesunate. On day 11, the clearance level was +3 which could be attributed to the antagonizing effect of vitamin A to artesunate.

Table 1: Effect of co-administration of Vitamin A and Artesunate on parasite level in P.berghei infected mice.

GROUP	5^{TH} DAY	7^{TH} DAY	9^{TH} DAY	11^{TH} DAY
A	++	+++	++	+

B	+++	+++	++++	++++
C	++	+++	+++	+++
D	+++	+++	++	+++

n=4(mean rounded to nearest whole number)

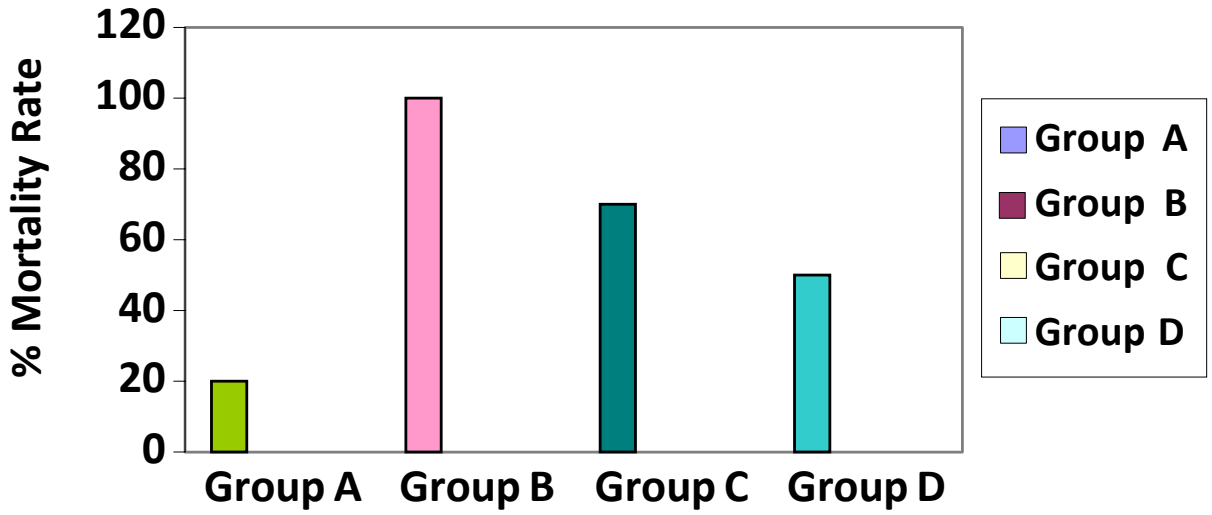

Fig. 1: Percentage (%) mortality of co-administration of Vitamin A and Artesunate in P. berghei infected mice.

Where: Group A = Artesunate + Parasite

Group B = Distilled water + Parasite

Group C = Vitamin A + Parasite

Group D = Vitamin A + Artesunate + Parasite

PACKED CELL VOLUME:

During the pre-inoculation period, in **Group A** is day 1 the PVC level was high and on day 5, there was a significant decrease on day 11, there was a slight increase in the PVC but not as same as on day 1 and this could be attributed to the administration of Artesunate building up the PCV level.

Group B, There was a significant decrease in PVC level on day 5 and also a decrease in the PCV level on day 11 which was lesser than day 5 which show more loss of PCV in the mice and it could be due to not having administration after the attack of parasitemia on the mice which is significantly lower when compared to Group A.

Group C, There was a significant decrease in PVC level on day 5 and also a decrease in the PCV level on day 11 which was lesser than day 5 which show more loss of PCV in the mice and it can be due to the no effect of Vitamin A on the parasitemia but it's PCV is a bit higher than Group B but lesser than Group A.

Group D, There was a significant decrease in PVC level on day 5 and also a decrease in the PCV level on day 11 which was lesser than day 5 which show more loss of PCV in the mice and it could be due to the antagonizing effect of Vitamin A to artesunate. The PCV level in Group D is slightly higher than Group B but significantly lower when compared to Group A and C.

Table 2: Packed Cell Volume for P.berghei-infected mice.

Group	1st Day (Pre-inoculation)	5th Day (Post-Inoculation)	11th Day (Post-Inoculation)
A	52.25±2.63	31.25±1.26	42.00±1.83
B	53.25±2.22	28.75±2.22	18.00±1.41
C	50.75±0.96	25.00±0.82	21.00±0.96
D	51.00±1.41	23.75±0.82	19.50±2.63

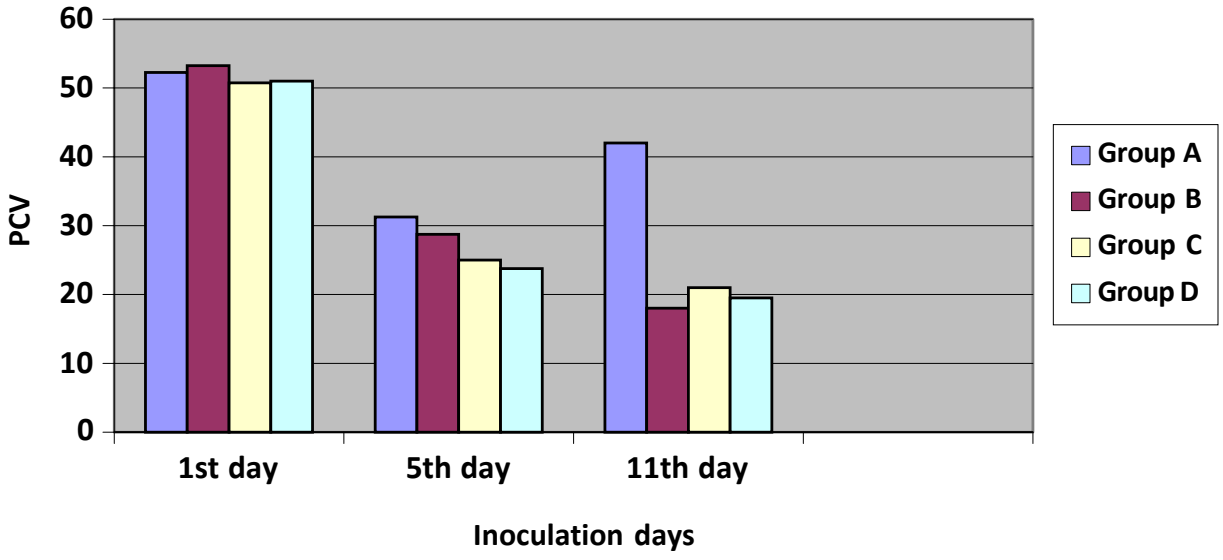

Fig. 2: Showing the Packed Cell Volume for the pre-inoculation day (1st day) and post-inoculation days (5th, 10th day) for P.berghei infected mice.

Where: Group A = Artesunate + Parasite

Group B = Distilled water + Parasite

Group C = Vitamin A + Parasite

Group D = Vitamin A + Artesunate + Parasite

BODY WEIGHT:

During the period of pre-inoculation of the Parasitaemia of P. berghei-infected mice, .all the experimental animals were observed to have normal physical activity. Weight changes were observed in the experimental animals, day 1 before Inoculation, and day 11 Post-Inoculation after administration of the experiment, with their weight differences. During the period of administration, **Group A** had a slight decrease in physical activities and ate a bit less than normal. There was a significant decrease in their weight when compared to the mean weight at pre-inoculation. **Group B** has the least weight when compared to other group and it could be a result of the parasitemia, **Group C and D** has a slight decrease in weight but not as severe as **Group B** and not better than **Group A** and it could be due to the administration administered.

Table 3: Weight for pre and post-inoculation for P. berghei infected mice.

Group	Mean ± standard deviation
All groups: Pre inoculation	28.00±1.71
Group A: Post Inoculation	26.25±1.23
Group B: Post Inoculation	15.25±0.50
Group C: Post Inoculation	19.50±0.58
Group D: Post Inoculation	17.25±1.50

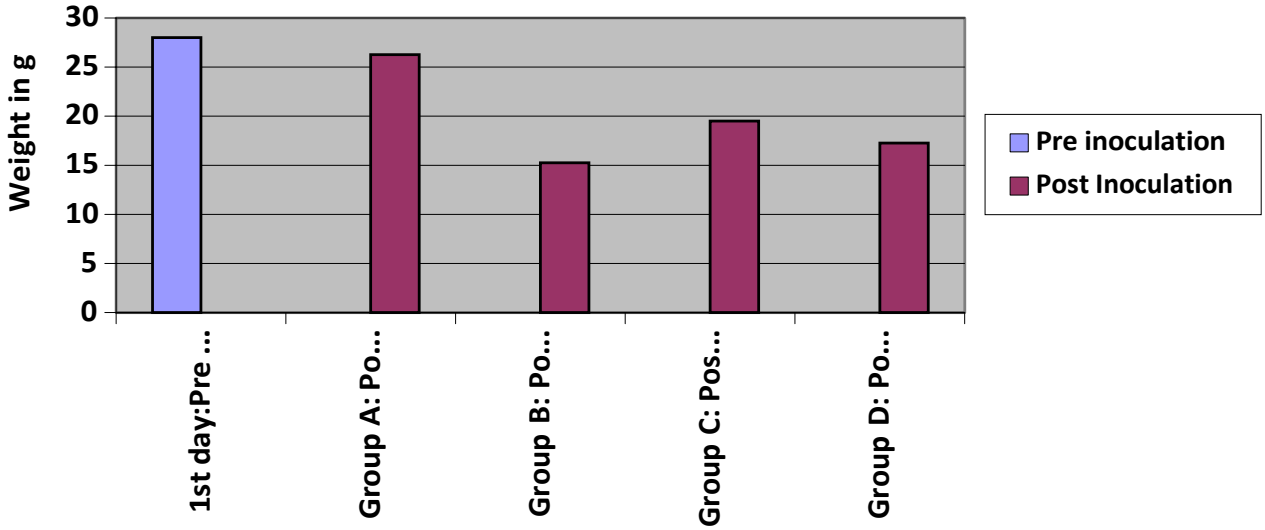

Fig. 3: Weight for pre and post-inoculation for P.berghei-infected mice.

Where: Group A = Artesunate + Parasite

Group B = Distilled water + Parasite

Group C = Vitamin A + Parasite

Group D = Vitamin A + Artesunate + Parasite

The above results show that Group A had the lowest Parasitaemia level, percentage mortality rate, increased weight, and PVC from the treatment of Artesunate as compared to the responses from the treatment of other Groups.

DISCUSSION

The findings of this investigation illustrate the intricate interaction between Artesunate and the antioxidant Vitamin A in mice infected with malaria. The study results indicate that the Parasitaemia level was significantly elevated as early as the 5th day following inoculation of the mice. The Parasitaemia level was predominantly within the range of +3(+++) prior to administering the drug. The life span of the mice was determined to be between the 6th and 11th-day post-inoculation.

The drug was administered to the mice on the 6th day following confirmation of parasitemia in this particular study. Upon administration of Artesunate and Artesunate in combination with Vitamin C to mice, parasitemia exhibited a decline and subsequent fluctuations across multiple groups beginning on day 5. As an established antimalarial drug with proven and validated efficacy, the study's findings were anticipated.

The parasitemia level of Group A observed to be +2 (++) on the 5th day, which gradually decreased to +1 (+) on the 11th day following the initiation of treatment with Artesunate. It was associated with a reduced mortality rate, as reported by Atanu F.O., *et al.,* in 2021, which demonstrated the efficacy of Artemisinin derivatives as potent antimalarial agents. A slight reduction in the body weight of the mice was observed, which may be attributed to the presence of paracetamol in their system, as reported by Dibessa T.T, *et al.,* in 2020. The administration of Artesunate has been observed to enhance the host's immune response against the parasite. This effect is believed to be mediated by generating free oxygen radicals in vivo. Our findings, as depicted in Figure 3, indicate that the use of Artesunate resulted in a significant increase in the body weight of animals compared to those in group D. Additionally, we observed an increase in the Packed Cell Volume in these animals. A notable reduction in packed cell volume was observed on the fifth day, followed by a slight increase on the eleventh day, attributed to Artesunate's administration. Severe malaria anemia (SMA) is a condition that often leads to a significant reduction in hemoglobin levels, which can be life-threatening. Artesunate as a sole treatment for SMA may exacerbate this condition, as it does not promote an increase in hemoglobin levels and may even cause a mild reduction. The studies conducted by Jauréguiberry *et al.,* in 2014 and White *et al.,* in 2018 are of relevance to the field of medical research.

On the 11th day, Group B exhibited a significantly elevated level of Parasitaemia ranging from +3(+++) to +4(++++) and experienced mortality, potentially attributable to oxidative stress induced by the parasite. A marked reduction in Packed Cell Volume was also observed, which may indicate hemolysis and resultant anemia. The study was conducted by Lopes L.N., *et al.,* in 2016. The observed symptoms

included a significant decrease in body weight, suggesting a substantial impact on the animals. This ultimately resulted in a mortality rate of 100%, leading to the mice's demise.

The study observed that Group C elevated Parasitaemia level from +2(++) on the 5th day to +3(+++) on the 11th-day post-administration of Vitamin A. Additionally, the group displayed a high Packed Cell Volume on the 1st day of pre-inoculation, which subsequently decreased on the 11th day. This suggests that the antioxidant effect of Vitamin A on malaria oxidative stress may not be discernible during the infection. It is important to note that Vitamin A is not an antimalarial drug, and the study conducted by Chen G., *et al.*, in 2019 reported a mortality rate of 75%. According to Ebohor *et al.*, 2021, it was observed that the body weight of the animals exhibited a significant decrease. This decrease in body weight may be attributed to the fact that vitamin A, a non-antimalarial drug, does not bring about any changes in this regard.

The findings indicate that Group D experienced a modest decrease in Parasitaemia level, from +3(+++) on the 5th day to +2(++) on the 9th day, followed by an increase to +3(+++) on the 11th day. These results suggest that Vitamin A may have counteracted the antimalarial properties of Artesunate, resulting in a 50% mortality rate and decreased PCV compared to Group A (refer to Figure 1). According to Shija K.M., *et al.*,'s research conducted in 2020, it has been proposed that Artesunate, a pro-oxidant, exhibits antiparasitic activity by producing free oxygen radicals. The study also suggests that Vitamin A, an antioxidant, may counteract the effects of pro-oxidants, indicating the presence of pharmacological antagonism.

The comparative analysis indicates that the efficacy of Artesunate in combating the malaria parasite is superior in group A compared to the combined administration of Artesunate and Vitamin A in group D.

CONCLUSION

The study demonstrated that Vitamin A can reduce the efficacy of Artesunate when used in combination. Therefore, clinical efforts should be made to control the concurrent use of Artesunate and Vitamin A as this may affect the treatment goal in malaria therapy.

Ethical Approval:

Animal Ethic committee approval has been collected and preserved by the author(s)

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