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Antimalaria Potential of Ethanolic Leaf Extract of *Phyllantus muellerianus* in *Plasmodium Berghei* Infected Mice

[1]*Stanley Chijioke Eluu,* [1]*Augustine Okpani Oko,* [2]*Uduma Eke Osonwa,*
[3]*Ngele Kalu kalu and* [3]*Benneth Emeka Osegbo*

[1]Department of Biotechnology, Ebonyi State University, Abakaliki, Nigeria
[2]Department of Pharmaceutics and Pharmaceutical Technology, NnamdiAzikiwe University, Nigeria
[3]Biology Microbiology Biotechnology, Federal University, Ndufu-Alike, Ikwo, Abakaliki, Nigeria

Abstract: This study was carried out to evaluate the *in vivo* antimalarial activities of the ethanolic leaf extract of *Phyllantus muellerianus* on *Plasmodium berghei* infected mice. Each mouse was infected intravenously in the tail vein with 1×10^7 parasitized erythrocytes from an infected donor mouse. Three days later, the mice were divided into 5 groups. The groups were orally treated with the extract (100, 200, 300 mg/kg), Artemisinin (120 mg/kg) was given to the positive control and distilled water to the negative control group. Also, Liver enzymes assay was done and hematological parameters were determined. The result of the study showed that at doses of 100, 200 and 300 mg/kg of the crude extract significant ($p<0.05$) higher reduction in the level of parasitaemia in mice when compared with the untreated group meanwhile, in the infected untreated group, parasitaemia continued to increase. Furthermore, it was observed that following infection the serum level of ALT, AST and ALP increased significantly ($p<0.05$) which were restored almost to normality after treatment. Also, there was general decrease in PVC, RBC and hemoglobin while an increase in WBC was recorded following infection. However, there was increase in these hematological parameters after treatment. It is obvious from the obtained results that P. *muellerianus* holds great promise for the treatment of malaria especially in this era of increasing drug resistance.

Key words: Malaria · Resistance · Treatment · *Phyllantus muellerianus* · *Plasmodium* · *Red Blood Cells*

INTRODUCTION

Malaria is an endemic infectious disease that is wide spread in tropical and sub-tropical regions of the world and one of the deadliest infectious diseases of human, caused by protozoan parasite of the genus *Plasmodium* [1]. Malaria is an increasing worldwide threat mostly in Asia and sub-Saharan Africa. In 2017, it was reported that there were an estimated 216 million cases of malaria which is an increase of about 5 million cases over 2015. Mortality reached 445 000, a similar number to the previous year [2]. One of the reasons for continual increase in the global prevalence of malaria resulted from increasing resistance of the parasite to antimalarial drugs [3]. The rate at which malaria parasites have developed resistance to currently used antimalarial drugs is alarming [4] thus there is an urgent need for the discovery and the development of new effective and safe drugs. Plants have been important sources of new drugs and several medicinal plants continue to provide easily accessible alternatives to widely used antimalarials [5]. In Africa, medicinal plants play an important role in the medical system especially in Nigeria; however, plant materials remain an important resource to combat serious diseases in the world [6]. The effectiveness depends on the phytochemical compositions of these plants. The phytochemical content of the plant may inhibit one or more stages of *Plasmodium* life cycle. The use of traditional medicines for the treatment of malaria and other diseases continues to be a growing practice among many African families, despite the availability of orthodox method of treatment [7]. Various plants such the neem, pawpaw, lemon grass, *Phyllantus* have been widely used traditionally for the treatment of malaria. Also, *Phyllanthus* species is often used in the traditional system of medicine for various ailments including dropsy, diabetes, jaundice, asthma and

Corresponding Author: Okpani Oko, Department of Biotechnology, Ebonyi State University, Abakaliki, Nigeria.

bronchial infections [8]. In the Ayurvedic system of medicine, *P. muellerianus* is used in problems of stomach, genitourinary system, liver, kidney and spleen. It is bitter, astringent, stomachic, diuretic, febrifuge and antiseptic. The whole plant is used in gonorrhea, menorrhagia and other genital infections. It is also useful in gastropathy, diarrhea, dysentery, intermitted fevers, opthalmopathy, scabies, ulcers and wounds treatment. This study tested the *in vivo* antimalarial activity of *Phyllantus muellerianus* against drug resistant *P. berghei* parasite. In addition, its effect on hematological and biochemical parameters was also determined.

MATERIALS AND METHODS

Plant Material: Leaves of *P. muellerianus* plant were collected in April, 2017 from Farmland in Edehishieke in Ebonyi local Government Area of Ebonyi State, Nigeria. The plant was identified and authenticated by Mr. Nwankwo, a Plant taxonomist in the Department of Applied Biology of Ebonyi State University, Abakaliki, Nigeria.

Extraction: The dried and powdered leaf of *P. muellerianus* was continuously extracted in a Soxhlet apparatus with 70 % ethanol for 72h. The liquid extract obtained was concentrated in vaccum at 40°C using rotary evaporator. The extract was stored in a refrigerator at 4°C until used for experiment reported in this study. The dry ethanolic extract was dissolved in distilled water to make the stock solution from which the various doses administered were prepared for use by serial dilution.

Animals: Adult Swiss albino mice (20-30 g) of both sexes obtained from the animal house of the Department of Veterinary Medicine, University of Nigeria, Nsukka, were acclimatized for 7 days before commencing the study. The mice were conveniently housed under standard environmental condition at 22-25 °C. All mice had ad libitum access to commercial feed pellets and clean water throughout the study. All the animals were treated in compliance with the National Institute of Health Guide for care and use of laboratory animals [9].

Test Organism: A chloroquine sensitive strain of *P. berghei* (ANKA) was obtained from the National Institute of Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria and was maintained by sub-passage in mice and was used for the study to evaluate the antimalarial activity of the plant material used in this study. Each mouse was infected intravenously in the tail vein with 1×10^7 parasitized erythrocytes from an infected donor mouse.

Curative Studies: Evaluation of the curative potential of leaf extract was done according to the method described previously [10]. Each mouse was infected intravenously in the tail vein with 1×10^7 parasitized erythrocytes from an infected donor mouse on the first and was regarded as day 0. Three days later, the mice were divided into 5 groups (5 mice of each). The groups were orally treated with the extract (100, 200, 300 mg/kg), Artemisinin (120 mg/kg) was given to the positive control and distilled water to the negative control group. The treatment was carried out once daily for 3 days and parasitemia was then examined by microscopy of Giemsa stained thin blood smear.

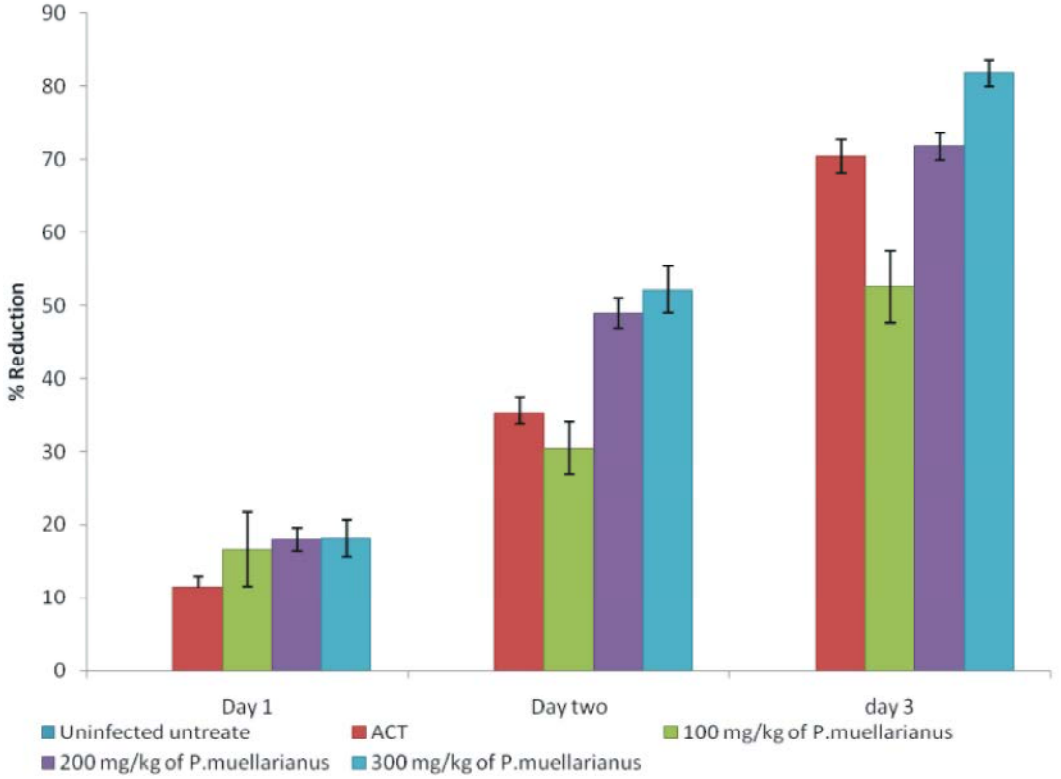
Biochemical Assay: Following treatment of the groups, the activities of liver enzymes were accessed for serum alanine aminotransferase (ALT) and alkaline phosphatase (ALP) using Randox kits according to the method described by Reitman and Frankel [11]. Briefly, blood samples were collected from all the animals before and at day 1 & day 2 post-treatment for further analyzes of AST, ALT and ALP using standard methods.

Hematological Parameters: The hematological parameters determined included: Packed Cell Volume (% PCV), Haemoglobin, Erythrocyte and Leukocyte counts and the determination was according to the methods described by [12].

Statistical Analysis: The results were expressed as means ± SD. significant differences between control and treatment groups were determined using one way Analysis of variance (ANOVA).

RESULTS

The *in vivo* assay revealed that the extract at 300 mg/kg more active than the ACT. At day one of treatment, the percentage of parasitaemia reduction was 11.46 % in group (E) treated with ACT and 18.11 % in group treated with 300 mg/kg (Fig. 1). At day two, percentage reduction in parasitaemia ranges from 70.39 % in group treated with ACT and 81.76 % in group treated with 300 mg/kg of the extract.



Significantly higher than ACT at $P < 0.05$

Fig. 1: Effect of leaf Extract of *P. muellerianus* on parasitaemia level in P. berghei infected mice

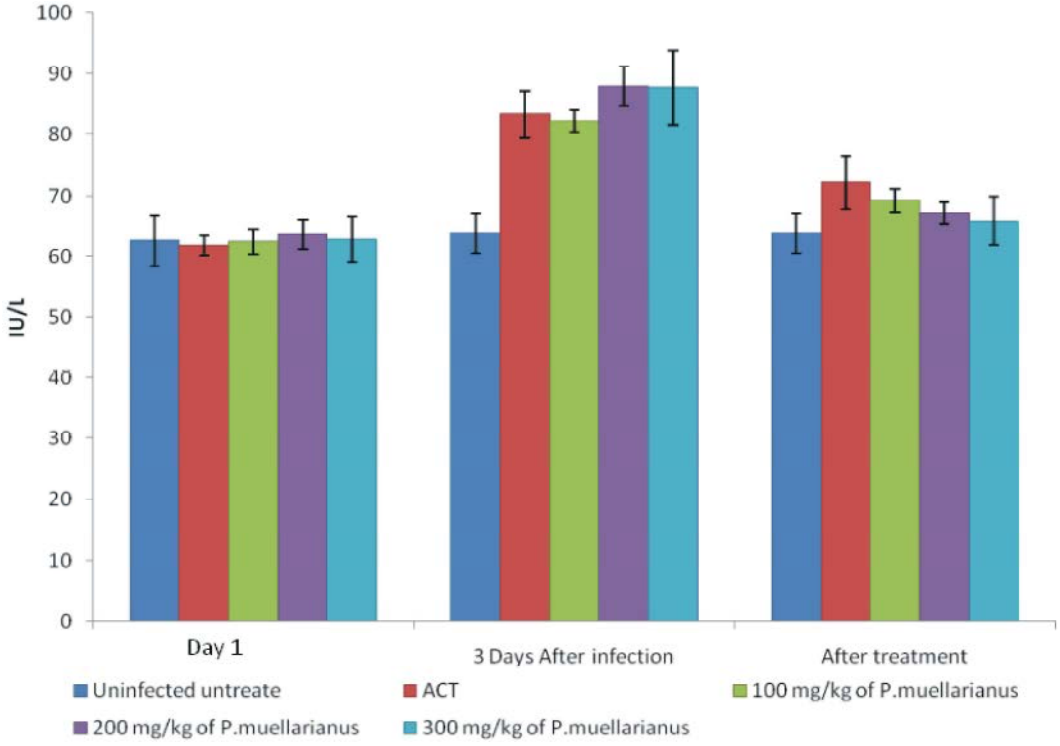


Fig. 2: Effect of treatment on serum ALT level of albino mice

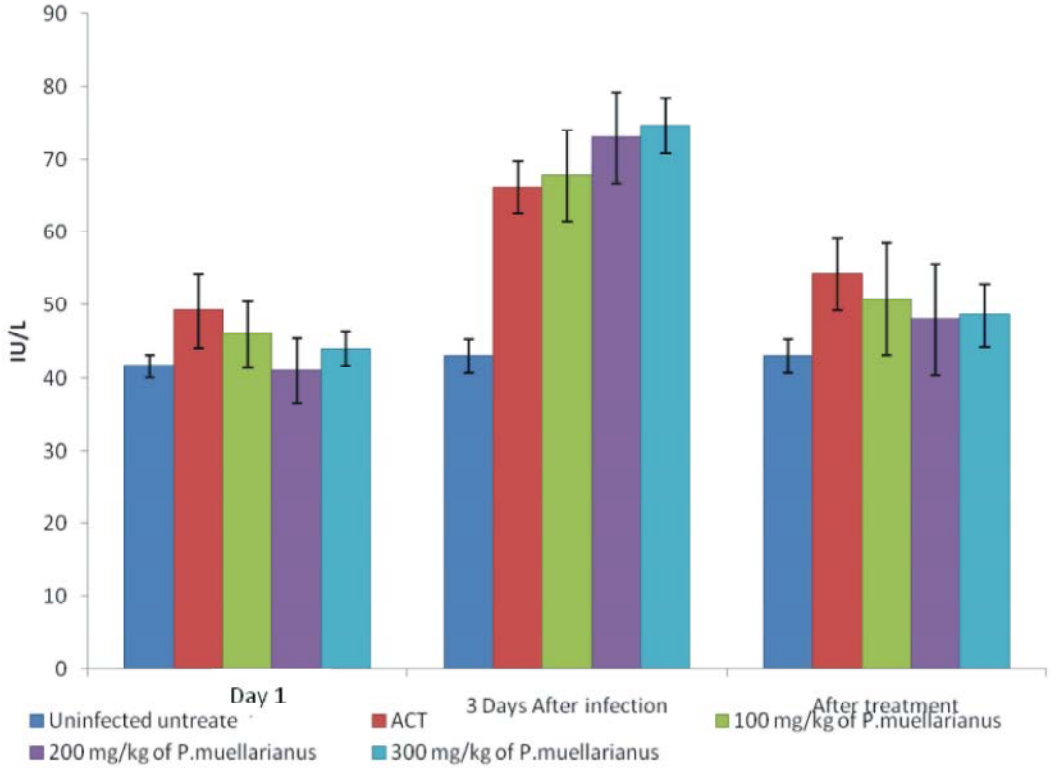


Fig. 3: Effect of treatment on serum ALP level of albino mice

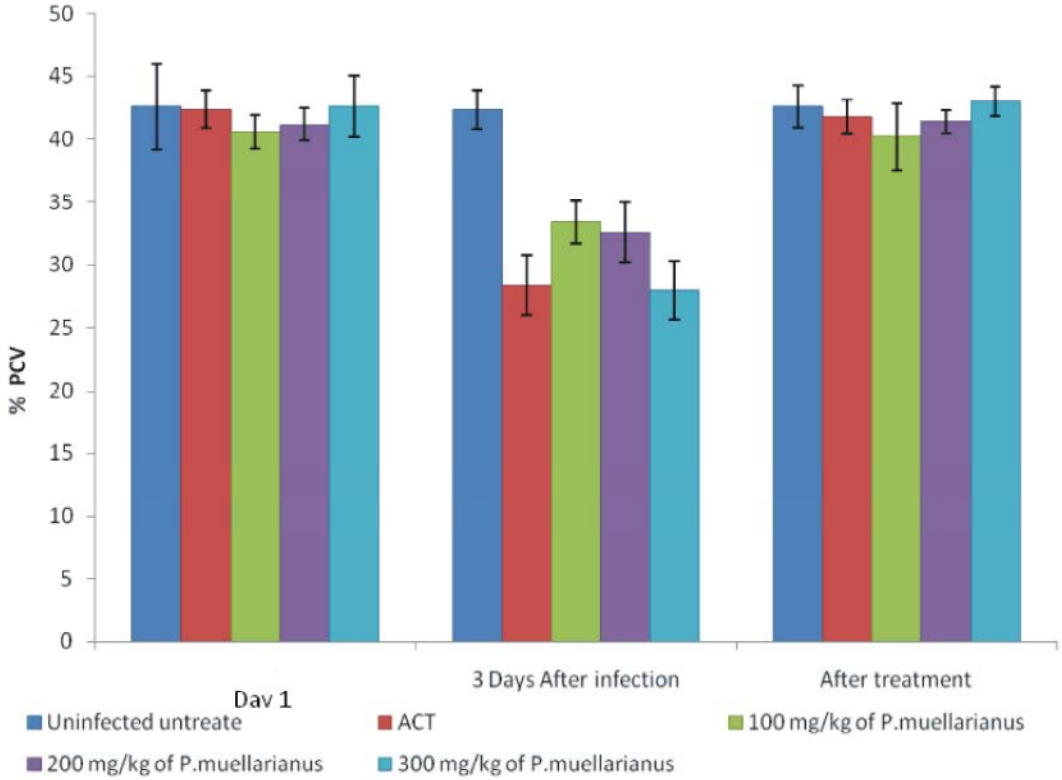


Fig. 4: PCV of group infected with *P. berghei* and treated with doses of *Phyllanthus muellerianus* leaf extract and ACT

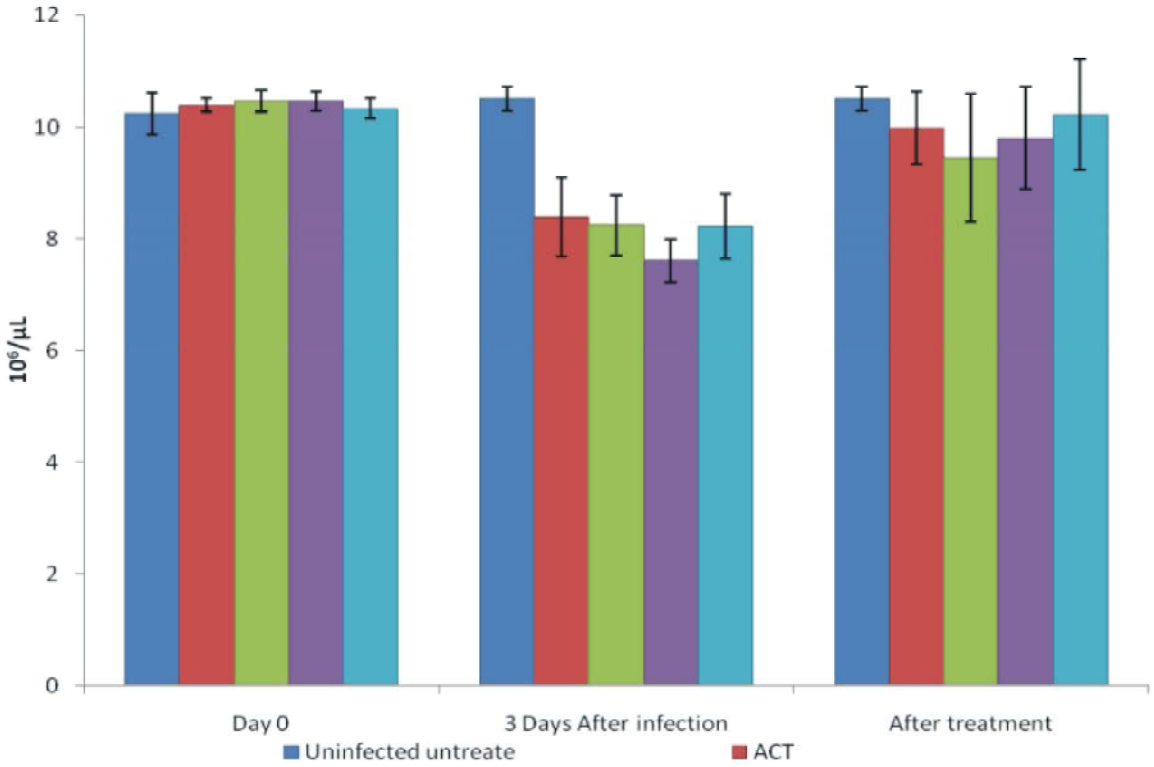


Fig. 5: RBCs of groups infected with *P. berghei* and treated with doses of *P. muellerianus* leaf extract and ACT

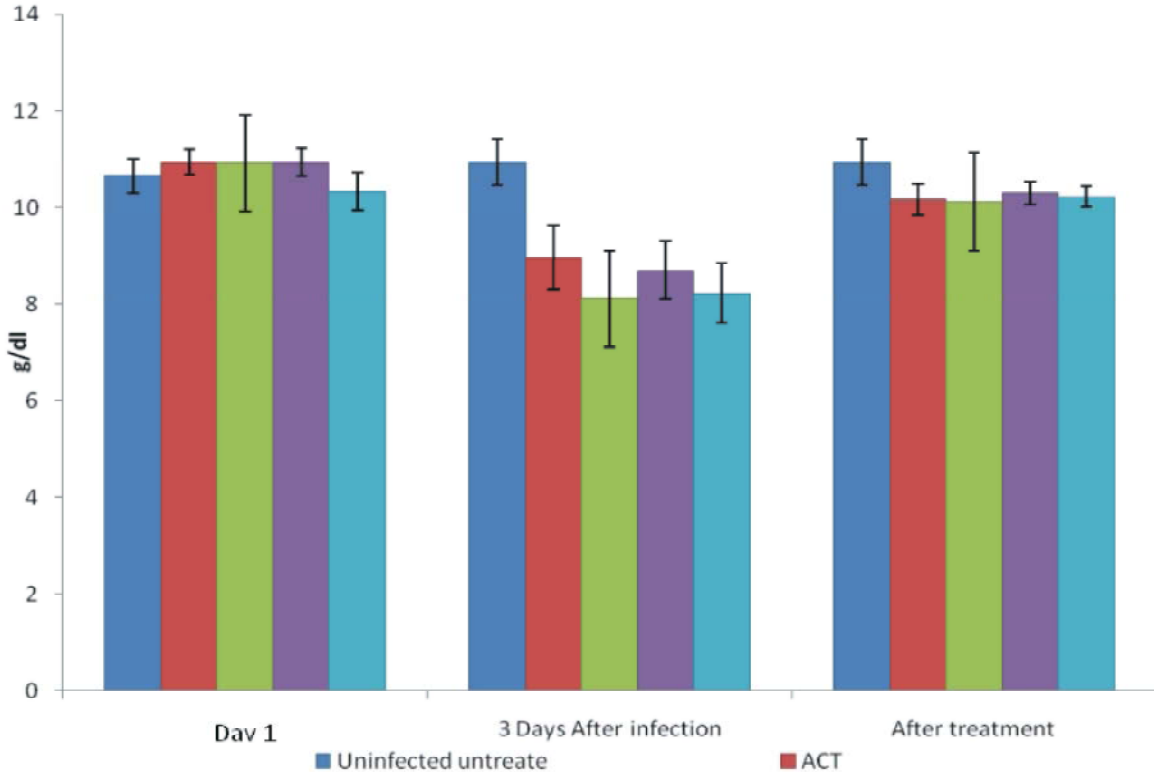


Fig. 6: Hb of rats groups infected with P. berghei and treated with doses of *Phyllanthus muellerianus* and ACT

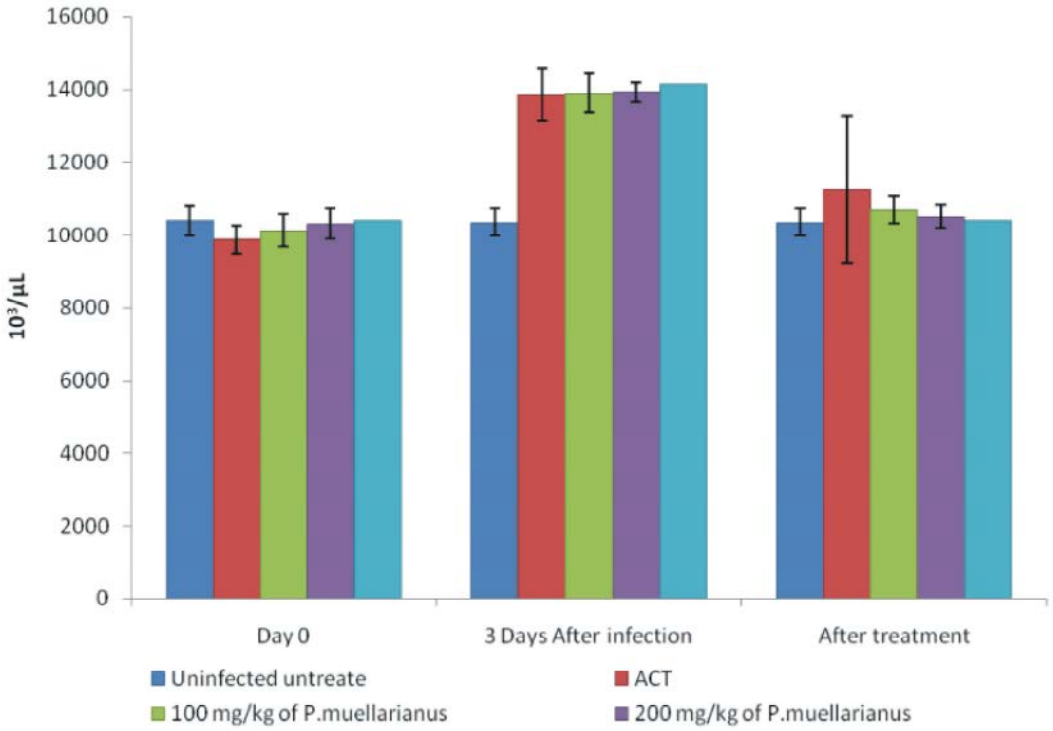


Fig. 7: WBCs of rats groups infected with P. berghei and treated with doses of *Phyllanthus muellerianus* and ACT

Biochemical test revealed that the mean ALT across the group is 32.6 ± 3.58 IUL^{-1} before infection. Three days after infection, the ALT was observed to increase to 50.2 IUL^{-1} except the uninfected group. Following treatment, there was a general decrease in serum ALT among the treated groups (ACT and the extract in a concentration dependent manner): ACT (from 50.6 $IUL^{-1}$to 40.6 IUL^{-1}), 100 mg/kg extract (50.0 to 39.8 IUL^{-1}), 200 mg/Kg extract (48.4 to 36.4 IUL^{-1}, 300 mg/kg extract (57.2 to 35.2 IUL^{-1}) while the uninfected group maintained 34.0 IUL^{-1} level (Fig. 2). Also, before infection the means ALP was 62.7 IUL^{-1}, then increase to 85.33 IUL^{-1} after infection. After treatment, the serum ALP decreased; the group treated with ACT decreased from 83.4 to 72.2 IUL^{-1} , 100 kg/mg extract from 82.2 to 69.2 IUL^{-1}, 200 mg/kg from 88. 0 to 67.2 IUL^{-1} and 300 mg/kg from 87.8 to 65.8 IUL^{-1}. There was significant difference ($p \leq 0.05$) in ALP level between the treatment groups three days after infection and after treatment. However, there was no significant difference ($p>0.05$) in ALP between the group before infection (Fig. 3).

The result of hematological assay showed a decrease in packed cell volume (PCV) on day 3 after infection. The use of ACT, 200 mg/kg and 300 mg/kg of the extract completely reversed the decrease caused by the parasites destruction of the PCV (Fig. 4). There was a general decrease in Red blood cell (RBC) count following infection which was restored after treatment. Increase doses of the extract showed a corresponding increase in RBC count although, ACT and 300 mg/kg of extract showed the same level of improvement after each treatment (Fig. 5).

The decrease in hemoglobin content after infection was restored after treatment but not completely to the initial level in group treatment with both ACT and the extract (Fig. 6).

The white blood cell count showed consistent increase from day1 of infection to day 3 from 1000 x 10^3/ml to above 12000 x 10^3/ml. Following treatment, each of the treatment group showed a decrease in WBC. The groups treated with ACT and 300 mg/kg of extract were restored to normal followed by the group treated with 200 mg/kg extract while the group treated with 100 mg/kg did not restore fully to its normal state (Fig. 7).

DISCUSSION

Phyllanthus species has been a very useful plant in the history of Ayurvedic and Unani system of medicine. Different parts of the Plant have been used widely in the treatment of stomach problems, genitourinary system, liver, kidney, spleen and Malaria [4].

The results of the present study revealed that ethanolic leaves extract of *Phyllanthus muellerianus* was able to significantly ($p<0.05$) reduce the percentage of parasitaemia in *P. berghei* infected albino mice in a dose dependent manner as seen in Fig. 1. The higher the dose of the extract, the higher the parasitaemia reduction. This is in accordance with the findings of Preeti *et al*. [13], who reported that leaf and root of *Phyllanthus* administered to *Plasmodium* infected albino mice showed antimalaria activities in dose dependent manner against the malaria parasite used in the study. There was significant difference in percentage reduction in parasitemia among the different treatment groups with the group treated with 300mg/kg showing the best results throughout the period of the study. The study also showed that the least reduction in parasitemia by the extract was observed on day one post treatment. It was also noted that even the lowest dose of the extract used in this study showed higher reduction in parasitaemia on the first day when compared to ACT. This suggests that the efficiency of the extract was higher than that of the standard drug, ACT at all the concentrations studied. The highest suppression of the parasites was observed in day 3 at the dose of 300 mg/kg of the extract which recorded 81.76 % which is higher than the group treated with ACT at day3 which recorded 70.39 %. This result is in line with the findings of Fahima *et al*. [14]. The study also demonstrated that continuous increase in the duration of administered extract can significantly ($p<0.05$) lead to decrease in parasitaemia as shown in figure 1. This is in line with the findings of Tolulope *et al*. [15], who in their study on anti-plasmodial effect of the extract and formulated capsules of *Phyllanthus amarus* on *Plasmodium berghei* infected mice, reported that high doses of the plant extract could be used as a substitute for the standard drug in the prevention of malaria infection.

Liver enzymes play crucial roles in normal cellular metabolism and when there is liver damage, these enzymes leak into the blood stream in excess amount. During *Plasmodium* infection, the sporozoites enter the liver cells through the blood stream and divide into merozoites [4-6]. These cause degenerative changes in the hepatocytes which are evidenced by increase serum level of the enzymes. Serum ALT and AST are useful indices for identifying hepatocellular damage and serum ALP for oestoblastic bone diseases and cholestatic hepatobillary lesion [16]. ALT measurement is more liver specific than AST and its activity is more than that of AST in acute hepatocellular damage [17, 18] while elevated level of serum ALT, AST and ALP indicate hepatic and cardiac cell damage [19, 20]. The study showed that 300 mg/kg of the extract had better effect on ALT restoration than the control drug. Also, the same concentration showed better restoration of AST and ALP than control drug. Since ALP measurement is more liver specific than ALP and, this result suggests that the extract is effective in treatment of liver damage caused by some disease conditions such as malaria, though in dose-dependent manner. However, the infected untreated group showed continuous increase in liver serum liver enzymes. This observed increase in ALT might be as a result of liver injury and altered hepatocyte integrity caused by the *Plasmodium* infection with the consequent release of the enzymes into the blood stream [21]. Anaemia has been a useful indication for malaria infection. Anaemia in the infected animals was characterized by significant reduction in the PCV, haemoglobin (Hb) concentration and RBC count. Malaria infection causes increase in the circulating WBC count. Following treatment with the extracts, depressed hematological parameters were restored to approximately normal. This could be as a result of high antiplasmodial effect of the extract against the parasitized red blood cells and the causative parasite, which sustained the availability of the new red blood cells produced in the bone marrow. Also, the elevated WBS was reduced to normal after each treatment. This observation suggests that the various concentrations of the extract studied as well as the control drug have effect on restoration of anaemia caused by malaria infection.

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