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Antimalarial Activity of Ethanolic Leaf Extract of *Sarcocephalus latifolius*and its Ameliorative Effect on Biochemical and Hematological Parameters of *Plasmodium berghei*-Infected Mice

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ABSTRACT

Malaria is a deadly disease that poses huge economic burden to the world most especially in the African continent. This study investigated the antimalarial activity of ethanol leaf extract of *Sarcocephalus latifolius*and its ameliorative effects on biochemical and hematological parameters of *Plasmodium berghei*-infected mice.The antimalarial assay was carried out using the method described by Akuodor *et al.*, in mice infected with chloroquine-resistant *Plasmodium berghei* ANKA strain. Animals were treated with 50, 100, and 150 mg/kg of ethanolthe extract as well as ACT which served as the standard drug. The activities of serum aspartate aminotranferase (AST), alanine aminotransferase (ALT), and alkaline phosphotase (ALP) were determined using randox kit method while hematological analysis was conducted using standard methods. The ethanol extract of *S. latifolius*significantly (p<0.05) inhibited parasitemia in a dose-dependent manner. In addition, there were no significant differences between the groups treated with 100 and 150 mg/kg of the extract when compared with the group treated with the standard drug.Furthermore, the results showed that the group treated with the extract exhibited significant (p<0.05) improvement in RBC count compared to the infected untreated mice. Significant reduction (p<0.05) in serum ALT, AST and ALT were noted mainly in higher doses of *S. latifolius*treatment and standard drug when compared to the infected untreated group. Individual variation in the level of ALT was noticed among the study groups. Therefore, this plant may be useful in the development of a new drug for malaria treatment owing to its high antimalarial activity.

Keywords:*Sarcocephalus latifolius,* malaria, *Plasmodium,* haematological, extract, antiplasmodial.

INTRODUCTION

Malaria is an endemic infectious disease that is wide spread in tropical and subtropical regions of the world and has almost certainly caused more morbidity and deaths than any other infectious disease, mostly in children under 5 years,

particularly in the developing countries of the world [1]; [2]; [3]. Greater number of the cases occur in Africa (78%) followed by Southeast Asia (15%) and Eastern Mediterranean regions (5%) [4]. Despite considerableattempt to control malaria in the last few decades, it remains a serious public health concern [5].

Currently, multi-drug resistance is one of the major problems hampering malaria control efforts [6], [7]. *Plasmodium falciparum*, one of the deadliest malaria parasiteshas developed resistance to all the classes of antimalarial drugs, includingartemisinin-based combination therapy(ACT) have been developed.It is widely reported that artemisininderivatives have distinct mode of action and remain the anti-malarial regimens of choice, though recentemergence of resistance to this monotherapy and ACTmay destroy this malarial treatment policy standard [8].

Although an effective vaccine would bethe best longstanding control option for malaria, current research on vaccine development is still at infancy and taking into account the different stages of vaccine development, it is estimated that a dependable malaria vaccine is several years ahead. In view of these problems, new treatment options are urgently needed. This has prompted research towards the discovery of new, safe and affordable antimalarial agents, mainly from plant sources [9], [10].In Africa and elsewhere, plant extracts are still widely used in the treatment of malaria and other ailments, and up to 80% of the African population use traditional medicines for primary health care [11].

The use of plants as natural remedies by mankind started from the very beginning of civilization [12] and a large number of modern drugs that are used today originated from natural sources.*S. latifolius*(also known as the African peach) belongs to the plant family *Rubiaceae*. It is a multi-stemmed tree or shrub up to 12meters high found in undisturbed fringing forest and close savannah woodland (Hutchison and [13]. In Nigeria, it has been observed that herbalistsuse this plant in the treatment and management of many ailments such as febrile illness, stomach disorder, cough, malaria fever and jaundices [14], hence the need for this scientific report on antimalarial activity of *S. latifolius*

MATERIALS AND METHODS

COLLECTION AND IDENTIFICATION OF PLANT

Fresh leaves of *S. latifolius* were sourcedfrom Umuoghara in Ezza-North Local Government Area of Ebonyi State, Nigeria and authenticated by Mr. Nwankwo, aplant taxonomist, in Applied Biology Department, Faculty of Science, Ebonyi State University, Abakaliki, Nigeria.

Extraction

The dried and powdered leavesof *S. latifolius* was continuously extracted in a Soxhlet apparatus with 70 % ethanol for 72h. The liquid extract obtained was concentrated in vacuum at 40°C using rotary evaporator. The extract was stored in a refrigerator at 4°C until used for the experiment reported in this study. The dried ethanolic extract was reconstitutedin 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 (25-30 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 [15]

Drug

Artemether- Lumefantrine an artemisinin combination drug consisting of artemether 20 mg and Lumefantrine 120 mg (CIPLA LTD, INDIA) was obtained from Godal Pharmacy in Abakaliki, Ebonyi State.

Parasite Inoculation

Malaria parasite, *P. berghei* (ANKA strain) was obtained from the Department of Veterinary Medicine, University of Nigeria Nsukka, and the strain was maintained by serial blood passage from mouse to mouse intraperitoneally.

Parasitemia determination

On day one, twenty-five out of the thirty mice were passagedintraperitoneally with infected blood suspension (0.2mL) containing $1x10^7$ *P. berghei* parasitized red blood cells.Seventy-two hours later, the rats were randomly divided into six groups of five mice per cage as follows: A, B, C, D, E and F. Group A was uninfected and administered normal saline hence served as the positive control, group B was infected and given normal saline (negative control), Group C was infected and treated with 5mg/kg standard drug (ACT) and served as normal control. Groups C, D and E were infected and treated with 50 mg/kg, 100 mg/kg and 150 mg/kg of the extract respectively.Treatment lasted for seven days and blood was sampledfrom the tail of each of the animals in each group using clean, non-greasy slides before and after treatment. Thin films were made accordingly and allowed to air-dry [16]. The films were fixed with methanol, stained with Giemsa and parasitemia density examined by microscopically counting the parasitized red blood cells on at least 1000 red blood cells in 10 different fields [17].

Hematological Parameters

The hematological parameters determined included: Packed Cell Volume (% PCV), Hemoglobin, Erythrocyte and Leukocyte counts, and the determination was carried out according to the methods described by [18]

Biochemical Parameters

Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were determined as described by [19] using assay kits (Randox Laboratories Ltd, UK). Serum alkaline phosphatase was determined as described by [20].

Statistical Analysis

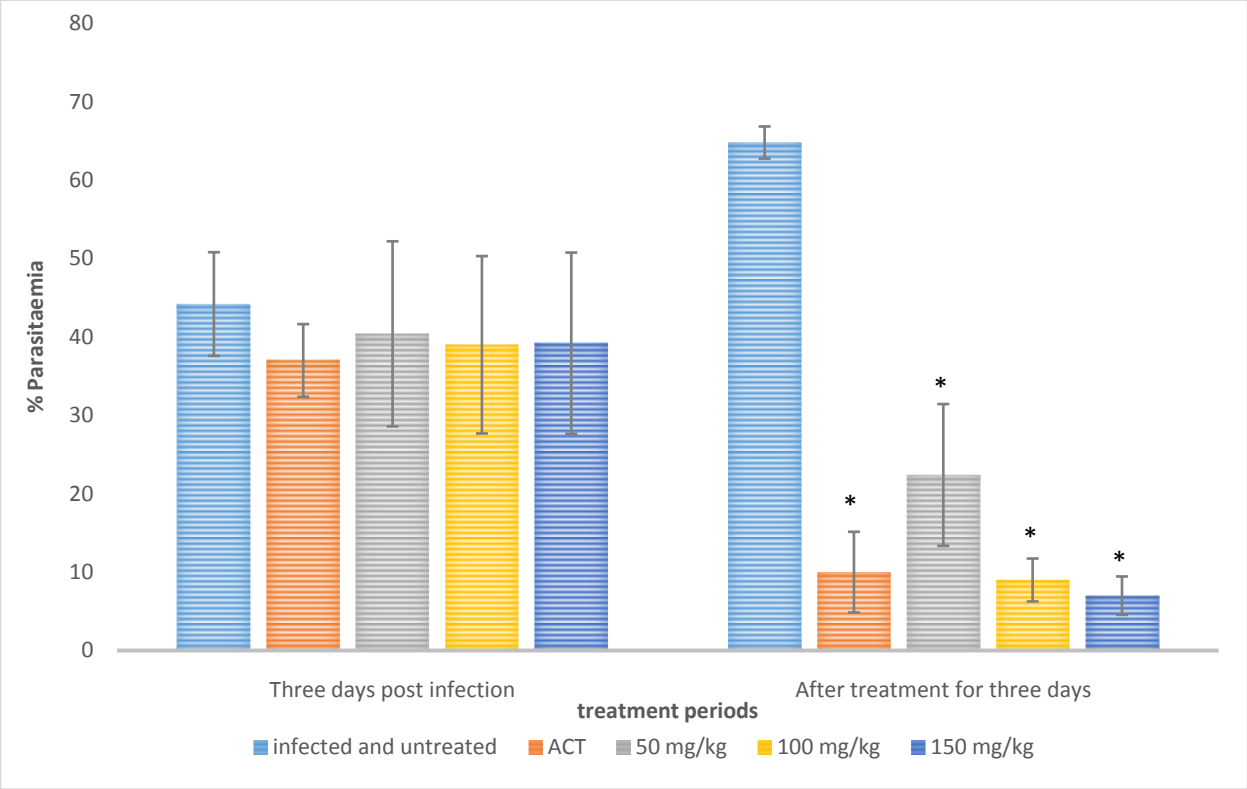
The results were expressed as mean ± standard deviation (SD). Parameters in the groups were compared by one-way (ANOVA) using the computer software Statistical Package for Social Sciences (SPSS) Version 20.0. All data was analyzed at 95% confidence interval and values were considered statistically significant at $p \leq 0.05$.

RESULTS

Effect of the Ethanol Leaf Extract of *S. latifolium* on Parasitaemia of *Plasmodium berghei* Infected Mice.

The results obtained in this study revealed significant decrease ($p \leq 0.05$) in % paritaemia seven days after treatment. The significant decrease ($p \leq 0.05$) in parastemia of the groups treated with *S. latifolius*observed in this study was dose dependent.However, there were no significant differences between the groups treated with 100 and 150 mg/kg

of extract when compared with the group treated with the standard drug. The treated groups also showed significantly higher reduction ($p>0.05$) in % parasitaemia when compared with the negative control (infected and untreated). In addition, it was noted that three days' post infection, there was no significant difference in % parasitaemia among all the groups indicating that the animals were of the same health status prior treatment.

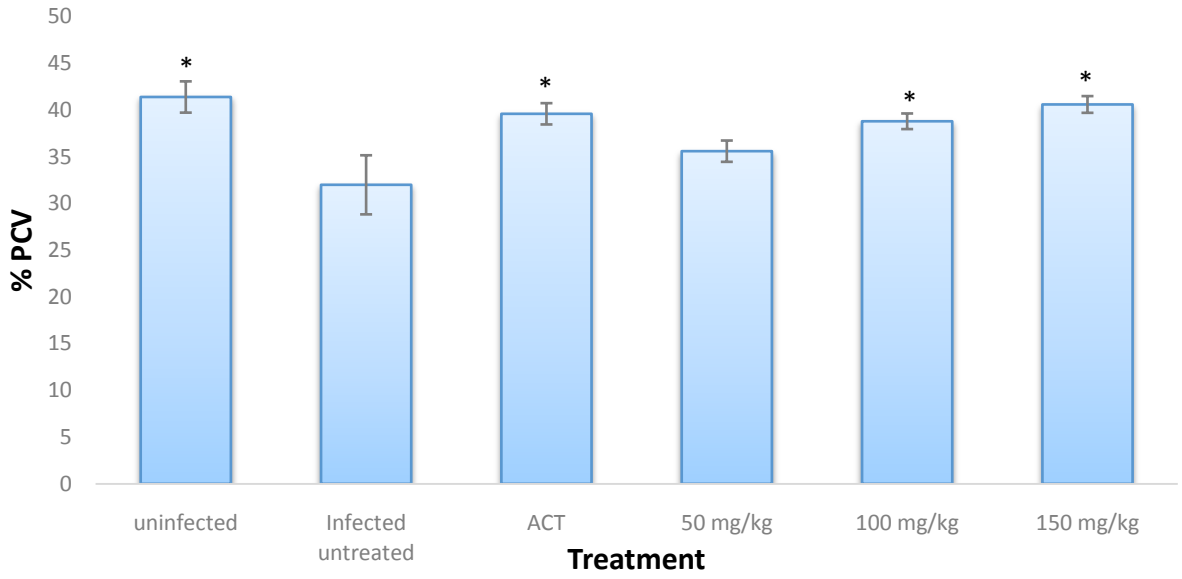


***Significantly lower than infected untreated animals at p≤0.05**

Figure1: Antiplasmodial effect of ethanol extract of *S. latifolius* leaf on *P. berghei* infected mice.

Packed Cell Volume

The result of the study showed that the uninfected mice (group A) had the highest % packed cell volume (41.4±1.67), followed by group F which was treated with 150 mg/kg of the extract (40.6±0.89), group C which was treated with the standard drug (39.6±1.14), group E, treated with 100 mg/kg of the extract (38.8±0.84), group D which was treated with 50 mg/kg of the extract meanwhile, Group B which was infected but untreated recorded the lowest PCV count (32±3.16) as shown in Figure 3. It was also noted from the result obtained that there was significant difference ($P\leq0.05$) in the PCV count of the different treatment groups when compared to the infected untreated group. However, there were no significant difference ($P>0.05$) in % PCV of the treated groups. Also, the result showed that PCV increased with increase in the concentration of extract administered indicating that effect of the extract was concentration dependent.

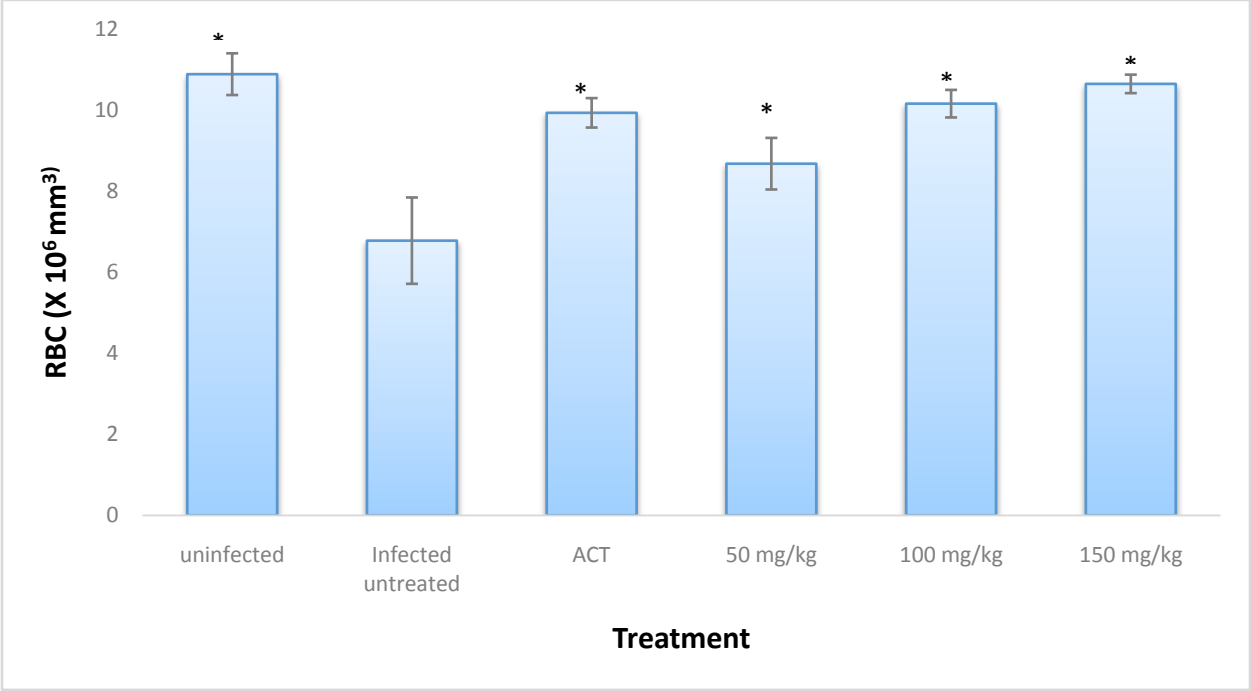


***Significantly higher than infected untreated animals at p≤0.05**

Figure 2: Effect of *Sarcocephalus latifolius*ethanol extracton Packed cell volume of mice

Red Blood Cell Count

The results showed that the group treated with the extract exhibited significant ($p<0.05$) improvement in RBC compared to the infected untreated mice. Also, the ACT treated group showed similar result. However, there were no significant differences ($p>0.05$) in RBC values among the treated groups.

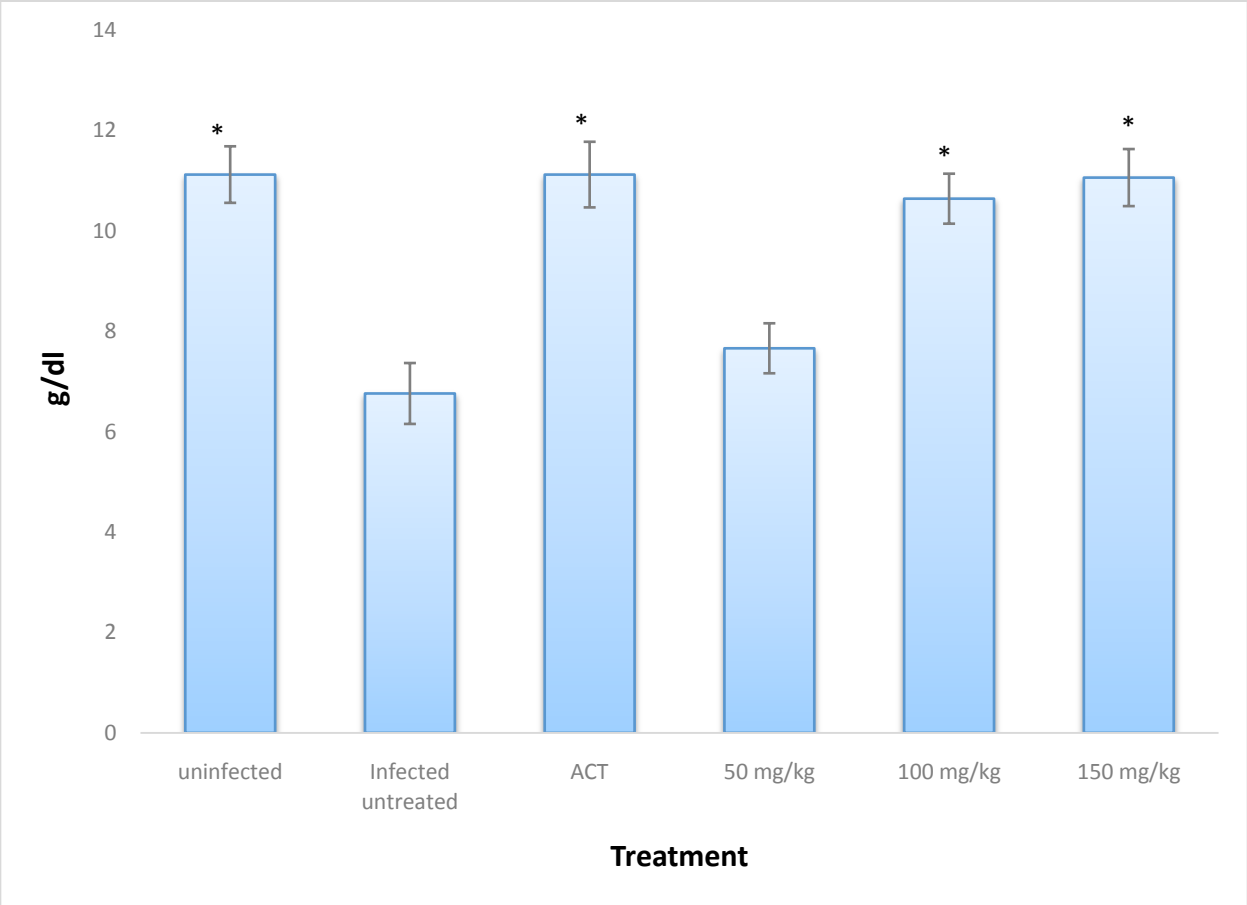


***Significantly higher than infected untreated animals at p≤0.05**

Figure 3: Effect of *Sarcocephalus latifolius*ethanol extracton Red blood cellcountof mice

Hemoglobin Count

Figure 4 shows that there were no significant (p>0.05) differences in hemoglobin content of the various groups treated with 50, 100 and 105 mg $kg^{-1}$of the extract when compared to the group treated with the standard drug. Also, the result showed that Hb increased with increase in the concentration of extract administered indicating that effect of the extract was concentration dependent. However, there was significant difference (P ≤0.05) in Hb count of the treated group when compared to the infected group

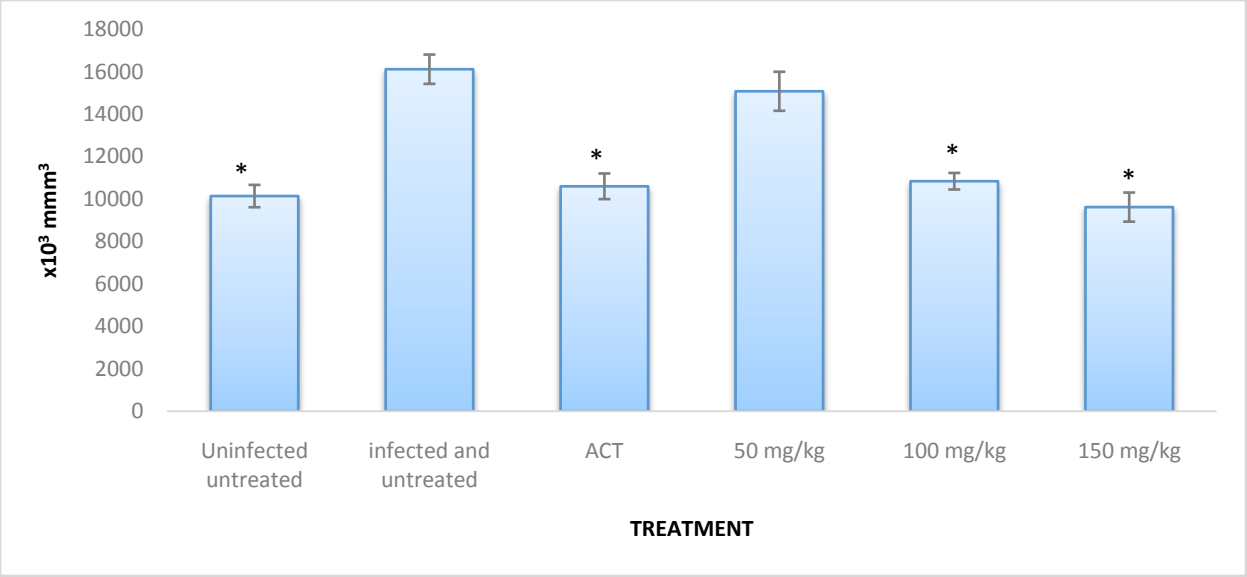


***Significantly higher than infected untreated animals at p≤0.05**

Figure 4: Effect of *Sarcocephalus latifolius* ethanol extract on Hemoglobin concentration of mice.

White Blood Cell Count

The result of the study showed that WBC count was highest in group B (infected and untreated) with values of 16120±690.65, followed by group D (treated with 50 mg/kg of extract) with WBC of 15080±920.33, group E, treated with 100 mg/kg of the extract which recorded WBC of 10840±391.15, group C which was treated with ACT with values of 10600±604.15, group A which was uninfected and untreated 10140±527.26) while the rats in group F which was infected but treated with 150 mg/kg of the extract recorded the lowest WBC count (9620±687.02 x10) as shown in figure 5.



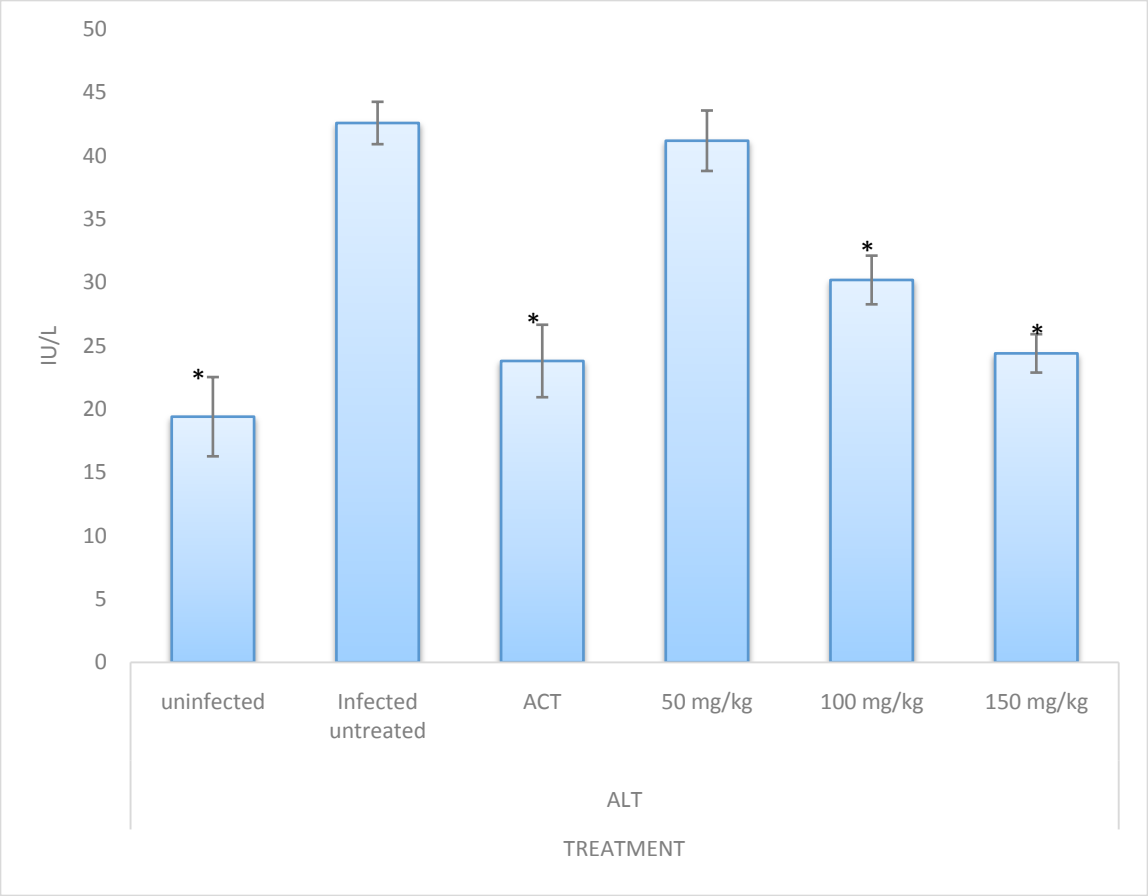
***Significantly lower than infected untreated animals at p≤0.05**

Figure 5: Effect of *Sarcocephalus latifolius* ethanol extracton White Blood cell count of mice

Biochemical Parameters of Mice

Alanine aminotransferase (ALT)

Significant reduction ($p<0.05$) in serum ALT were noted mainly in higher doses of *S. latifolius*treatment and standard drug when compared to the infected untreated group. Individual variation in the level of ALT was noticed among the study groups. Serum ALT levels were significantly reduced ($p<0.05$) by the intake of *S. latifolius*in a dose dependent manner.

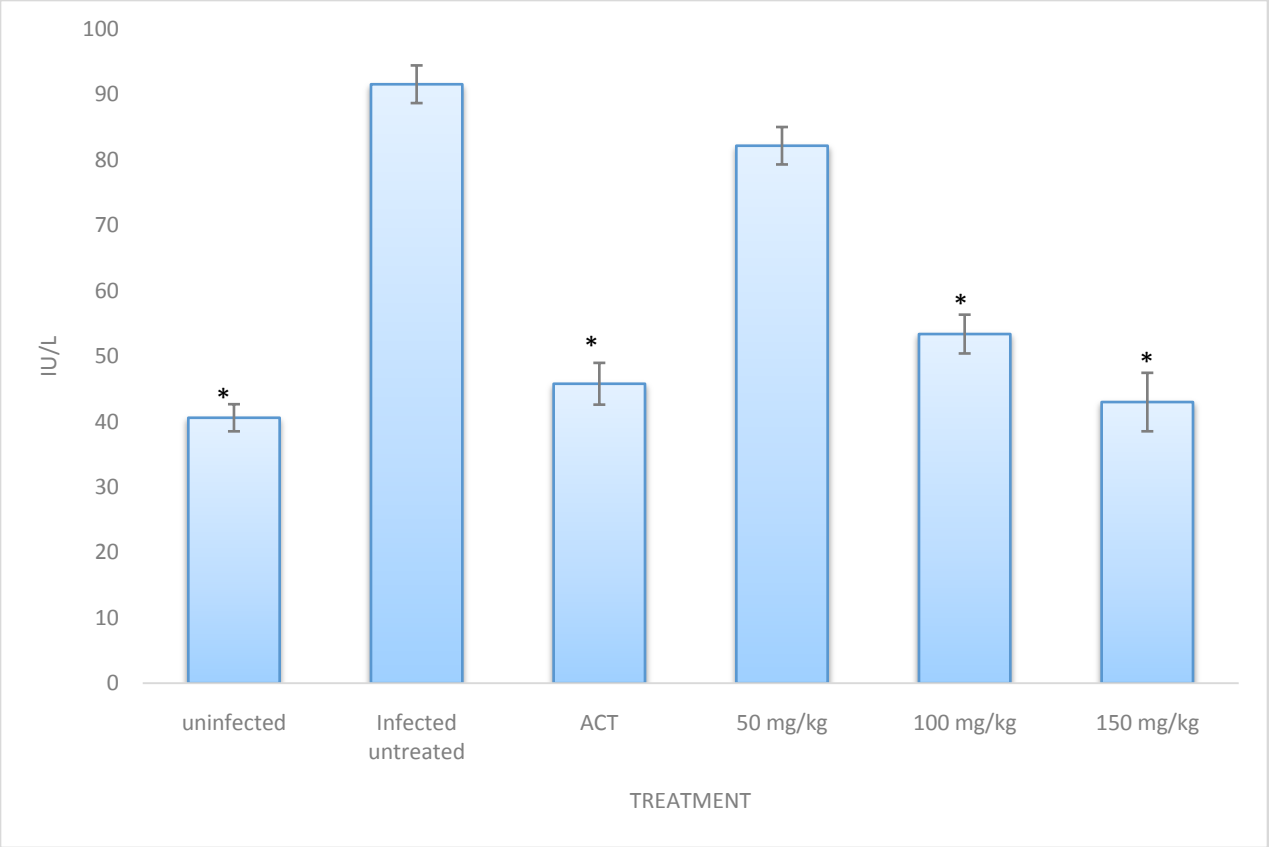


***Significantly lower than infected untreated animals at p≤0.05**

Figure6: Effect of *Sarcocephalus latifolius*ethanol extracton Alanine aminotransferase of mice

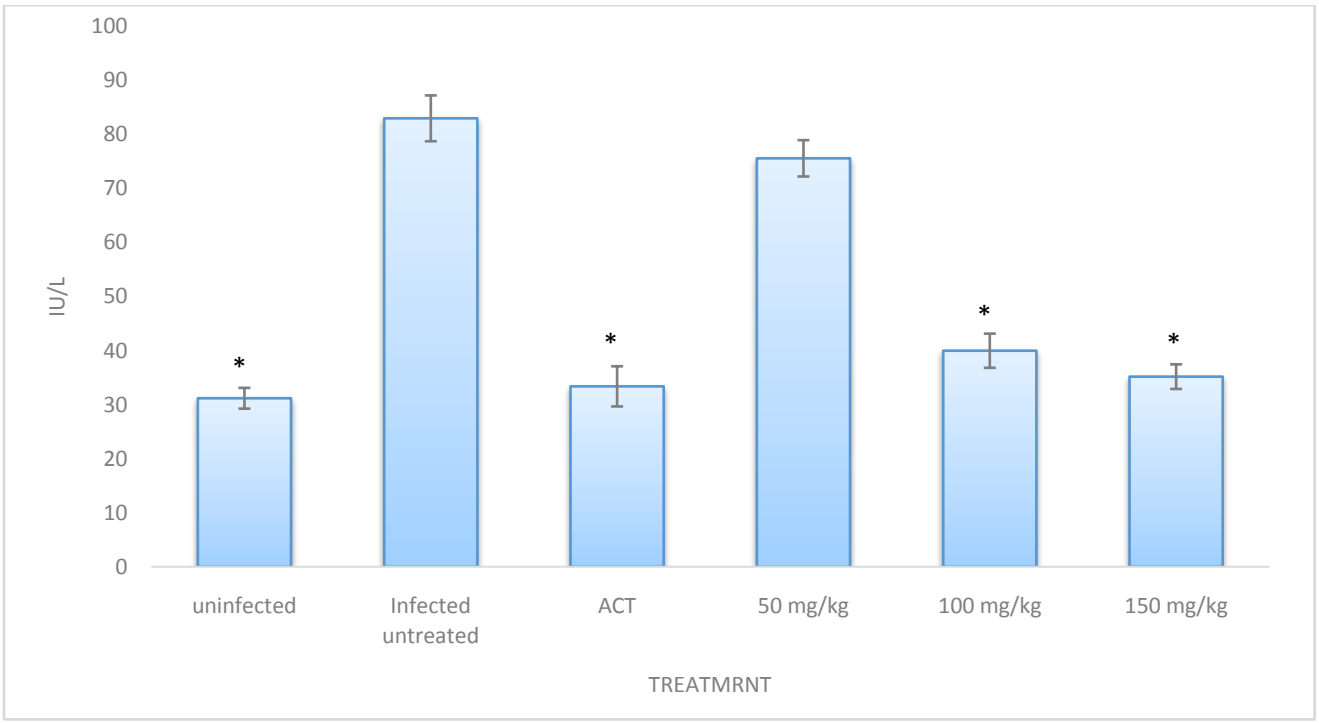
Aspartate aminotransferase (AST)

There was significant decrease ($p<0.05$) in the activities AST in the treated animals when compared to the infected untreated group. However, the uninfected untreated group had no significant difference ($p>0.05$) in serum AST when compared to the group treated with the standard drug,100 and 150 mg/kg of extract.



***Significantly lower than infected untreated animals at p≤0.05**

Figure7: Effect of *Sarcocephalus latifolius*ethanol extractonaspartate aminotransferase of mice



***Significantly lower than infected untreated animals at p≤0.05**

Alkaline Phosphatase (ALP)

Figure 8 shows a dose dependent decline in the alkanine phosphatase activity in the treated groups:Uninfected-untreated(31.20±1.92), Infected-untreated (83.0±4.24), ACT (33.40±3.72), 50mg/kg (75.6±3/36), 100mg/kg (40.0±3.16) and 150mg/kg (35.2±2.28).

DISCUSSION

The antimalarial activity of the plant extract was investigated using *P. bergei* infected mice. *In vivo* models are normally utilized in antimalarial studies owing to the fact that they take into account the potential prodrug effect and possibleparticipation of the immune system in elimination of the pathogens [21]. The antiplasmodial activity displayed by the extract was comparable to the standard drug used. The observed effect also showed direct proportionality with the dose of the extract administered such that the higher doses produced higher parasitaemia suppression. This dose dependent reduction in the parasite level observed is in line with the reports of [22] and [23]. The high level of antimalarial activity exhibited by *S.latifolius* in this study (Fig.1) may be due to the presence of phytochemicals. Phytochemicals constitute an indispensable part ofmedicinal plants and are responsible for their innumerable bioactivities. The leaf of *S. latifolia* have been reported to contain different phytochemicals including tannins, alkaloids, flavonoids, anthraquinone, Glycoside and terpenoids [24]. Flavonoids, polyphenols, saponins, alkaloids have been documented to elicit antimalarial activity [25]; [26].Thenoted antimalarial activity of *S.latifolius*is consistent with thetraditional use of plant as herbal medication for the treatment of malaria. It is worthy of note thatthe minimumantiplasmodial effect recorded by the group treated with 50 mg/kg of the

extract may have resulted from short duration of action of the extract occasioned by rapid metabolism which prevented complete parasite clearance. However, the significant increase in the level of parasitaemia of the infected-untreated group compared to the treated groups (Fig.1) correlates with the view that parasitemia increases progressively after infection until the point of death in the absence of suitable treatment [27].

An assessment of the impact of the extract on hematological parameters were carried out in this studyowing to the fact that malaria parasite infection causes prominent changes in blood and blood-forming systems [28]. Anaemia is usually appraised by evaluating the packed cell volume (PCV), haemoglobin (Hb), and red blood cell (RBC) count in malaria infected people [29]. However, estimation of haemoglobin concentration is fundamentally used to determine the presence of anaemia or, its reverse, polycythaemia [30]. The packed cell volume indicates the potential of blood to transport oxygen and nutrients [31] while hemoglobin has the physiological function of carrying oxygen to tissues of the animal for oxidation of ingested food so as to release energy for proper functioning of the body [32]. The significantly low level of hemoglobin, PCV and RBC observed in the infected untreated group when compared to the other groups is consistent with anaemia seen in malaria. This observation corresponds with the findings of [33] who reported decrease in hematologicalindices of rabbits infected with malaria parasite. This outcome is also supported by independent findings of [34]; [35]; [36]. However, the hematological parameters such hemoglobin concentration, hematocrit value and RBC indices were restored following treatment with *S. latifolius.* The increased hematological indicesindicate a better transportation capacity of the red blood cells and this may be due to the antimalarial properties of the extract. Moreover, the increased WBC count of the infected-untreated group observed in this study can be linked to the malaria infection [37]. This may be due to the production of white blood cells by the animal to fight against the invading pathogen. White blood cells are the cells of the immune system that are involved in protecting the body against both infectious disease and foreign invaders [38].However, following treatment, the WBC count was lowered indicating the destruction of the parasites due to treatment which lead to reduced production of WBC.

Determination of enzymatic activities in tissues plays an important role in the diagnosis and evaluation of liver damage [39]. AST and ALT are biomarkers of hepatic integrity and to a certain level can be used to measure the magnitude of hepatocellular damage; the ALT activities however, give more valuable information relevant to the integrity of the hepatocyte than AST [40]. The increase in serum ALT, AST and ALP activity observed in the infected untreated animals noted in this study may indicate hepatic damage probably by the altered cell membrane permeability leading to the leakage of the enzymes from the tissues to the serum. These observations confirm the previous report that parasitic infectious gradually affect enzyme activities by causing elevation of serum activities of AST, ALT and ALP which is an indicationof liver damage [12]; [17]. [23]also reported that *Plasmodium* infection causes degenerative changes in the hepatocytes and consequent release of enzyme in the blood stream. However, it was noted that the levels of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and

Alkaline Phosphatase (ALP) in the treated groups reduced in the course of the study whichmay be attributed to the reduced rate of synthesis of the liver enzyme as a consequence of exposure to treatment. This is result is in line with the findings of [31] who reported reductions in ALT and AST activities following administration of *S. mombin* leaf extract.

CONCLUSION

In this study, we established that the ethanolic leaf extract of *Sarcocephalus latifolius* had significant and dose-dependent activity against P. bergei in infected mice and it also showed ameliorative effect on the biochemical and hematological parameters of the mice.

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