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In Vivo Antimalarial Screening of Ethanolic Extract of *Cassytha Filiformis* and its Ameliorative Effect on Haematological and Biochemical Parameters Altered in *Plasmodium berghei* infected Mice

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ABSTRACT

This study investigated the antimalarial effect of ethanolic extract of *Cassytha filiformis* and its ameliorative capacity on haematological and biochemical parameters altered in *Plasmodium berghei* infected mice. Acute toxicity study was carried out using the modified Lorke method 25 out of 30 mice were infected with *Plasmodium berghei*, randomly assigned into 5 groups of 5 mice each, treated with 100mg/kg, 200mg/kg, 400mg/kg body weight of the extract and 5mg/kg body weight of Artemisinin Combination Therapy (ACT). Parasitaemia was monitored for 3 consecutive days by microscopy. Haematological indices (RBC, PCV, Hb concentration and WBC counts) and biochemical parameters (ALT and AST) were analyzed at the expiration of treatment. Oral LD₅₀ of the extract was determined to be 2154mg/kg body weight of the extract. The antimalarial activity observed increased with increasing dose of the extract, although not significantly different ($p > 0.05$) and also comparable with that of standard drug. Haematological parameters investigated decreased significantly ($p < 0.05$) in the infected-untreated group compared to the groups treated with graded dose of the extract with the exception of WBC count which was significantly higher ($p < 0.05$) in the infected-untreated group. Serum AST and ALT levels increased significantly ($P < 0.05$) in the infected-untreated group compared to the extract treated groups. We report the antimalarial activity of ethanolic extract of *Cassytha filiformis* for the first time and recommend its use in phytomedicine.

Keywords: Antimalaria, *Cassytha filiformis*, Haematological, Ethanolic, *Plasmodium berghei*, Parameters, Extract, Biochemical.

INTRODUCTION

Malaria is the world's most important protozoan disease [1], caused by five protozoan species of the genus *Plasmodium*, namely: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. Knowlesi* [2]. In 2017, an estimated 219 million cases of malaria occurred worldwide, compared with 239 million cases in 2010 and 217 million cases in 2016 [3]. Despite the many and varying efforts at malaria

control, this disease is still a global threat of enormous proportion and a significant contributor to health and economic inequities in endemic countries [4]. The 10 African countries with highest burden reported increases in cases of malaria in 2017 compared with 2016. Of these, Nigeria, Madagascar and the Democratic Republic of the Congo had the highest estimated increases, all greater than half a

million cases [5]. Nigeria accounts for more cases and deaths than any other country in the world [6]. *Plasmodium falciparum* is the most virulent and is responsible for the majority of malaria related morbidity and mortality [7] with significant social and economic impact in developing countries.

Clinical resistance to artemisinin and its combinations was reported in some parts of the world (Cambodia and four other countries), suggesting that some *P. falciparum* isolates have developed the ability to grow in the presence of these antimalarial agents [8].

Therefore, the treatment of multi-drug resistant malaria has increasingly become a challenging task in most malaria endemic regions of the world, which necessitates the urgent development of newer and effective antimalarial drugs that would fight against resistant malaria [9]. In order to resolve the above challenging issue, plants and/or plant-based traditional medicines are believed to be the most reliable and alternative means for the discovery of new antimalarial molecules as nature always serves as the richest source of chemicals of pharmacological importance [10].

Cassythafiliformis is a leafless, climbing, twining, vine-like, autoparasitic and plant-hyperparasiticphanerogam (seed-bearing plant) in the plant family Lauraceae. It is one of many higher flowering plant

species that have, through evolutionary divergence, become parasitic on various organs of other higher plants. Having long ago lost certain metabolic processes and physical structures to support it and remain independent, *C. filiformis* clings to other, mainly woody plants for physical support, nutrition, and water worldwide [11]. It appears to be totally indiscriminate in host choice, often covering and parasitizing dozens of host species simultaneously [12] and serves as a potential biological control for invasive plants. It is medicinally used as antiplatelet, Vesorelaxant [13], alpha-adrenoreceptor antagonist and antitrypanosomal [14]. In traditional medicine, the plant is pounded and the water extracts used in the treatment of difficulty in urination. In modern medical research, *C. filiformis* extracts have been seen to have diuretic activity [15]. Phytochemical screening reveals that the aerial parts of *C. filiformis* consists of alkaloids, flavonoids, triterpenoid, and steroids. Other reports show that some of the isolated compounds from this plant are lignan, cassyformin, filiformin, apomorphine alkaloids, actinodophine and octeine [16]. The presence of these phytochemicals explains the antimicrobial/medicinal potentials inherent in the *C. filiformis* extracts [17]. Hence the need to investigate its antimalarial potentials.

MATERIALS AND METHOD

Collection and Identification of Plant Material

The plant *C. filiformis* was collected from Nkalahacommunity in Ishielu Local Government Area of Ebonyi State and identified by Dr. Nnamani, C. V. a plant Taxonomist in the Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria.

Extraction

The plant samples were sorted to eliminate unwanted particles, dried at room temperature for two weeks, grinded into powder using an electric mill, sieved and stored. 200g of the powder was extracted with the aid of a Soxhlet extractor using 600 ml of 99 % ethanol for 72 h. This was repeated until enough

quantity was gotten and concentrated to dryness using a Rotary Evaporator and the residue stored in a freezer at 4 °C. Prior to use, the extract was dissolved in distilled water so that the doses required were prepared by necessary dilutions and given according to weight of the animals.

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 [18].

Drug

P-Alaxin an artemisinin combination drug (consisting of dihydroartemisin 40 mg and Piperaquine phosphate 320 mg), manufactured by Bliss GVS Pharmaceutical LTD, India was obtained from Godal Pharmacy in Abakaliki, Ebonyi State, Nigeria and diluted in distilled water to final dose of 5 mg/kg body weight.

Parasites

The parasite, *Plasmodium berghei* (ANKA strain) was obtained from the National Institute for Pharmaceutical Research and Development, Abuja (NIPRD) and maintained by serial passage in mice.

Acute Toxicity Study

Acute toxicity of the plant extract was determined according to the method of [19]. In which the extract was tested on three groups of three (3) mice each using three doses administered once orally as shown below: 1600mg/kg, 2900mg/kg and 5000mg/kg corresponding respectively to Groups 1, 2 and 3. The animals were placed under observation for 24 hours to monitor their behavior as well as if mortality would occur. The LD₅₀ was determined as shown below:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D₀ = Highest dose that gave no mortality,

D₁₀₀ = Lowest dose that produced mortality.

Curative test

In this experiment, 25 out of 30 albino mice were intraperitoneally infected with blood suspension (0.2ml) containing 1 X 10⁷ *P. berghei* parasitized erythrocytes. Seventy-two hours later, the mice were randomly divided into six groups of five mice per cage and oral treatment commenced on day 4 post infection as follows: Group 1 (Uninfected Untreated): Animals in this group were

not infected with *P. berghei* and were given normal saline, serving as Positive Control, Group 2 was infected with *P. berghei* and treated with 100 mg/kg body weight of the extract. Group 3 was infected, treated with 200 mg/kg body weight of the extract. Group 4 was infected and treated with 400 mg/kg body weight of the extract. Group 5 animals were infected with *P. berghei* and treated with 5 mg/kg of ACT serving as Normal Control while Group 6 animals were uninfected and untreated with the extract but were rather administered normal saline hence serving as Negative Control. Treatment lasted for 3 days and each day blood samples were collected from each of the groups from the animal tail using clean, non-greasy slides before treatment. Thin films were made accordingly and allowed to air-dry. 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 [20].

Hematological Parameters

Hematological parameters such as Packed cell volume (% PCV), Hemoglobin count, Red blood cell and White blood cell counts were determined using the methods described by [21].

Biochemical Parameters

Determination of serum Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities were carried out according to the method of (Reitman and Frankel, 1957) using assay kits (Randox Laboratories Ltd, UK).

Statistical Analysis

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

RESULTS

Acute toxicity

Acute toxicity study of *C. filiformis* showed that Groups 2 and 3 administered

2900 mg/kg and 5000 mg/kg body weight respectively died after 24 hrs of treatment, unlike Group 1 treated with

1600 mg/kg that displayed no sign of toxicity. The Mean Lethal Dose LD₅₀ is therefore determined thus:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D₀ = Highest dose that gave no mortality = 1600 mg/kg

D₁₀₀ = Lowest dose that produced mortality = 2900 mg/kg

$$LD_{50} = \sqrt{(1600 \times 2900)} = 2154 \text{ mg/kg body weight of the extract.}$$

Antiplasmodial testing

Figure 1 shows the percentage reduction in parasite count of the different

treatment groups for the three days that treatment lasted. The treatment groups showed a significant increase (p<0.05) in percentage parasitaemia reduction when compared to the infected-untreated group. However, the percentage parasitaemia reduction increased with increasing dose of the extract, with the standard drug producing the highest effect though not significantly different (p>0.05) from those of the graded doses of the extract. These findings followed similar pattern within the three days of treatment.

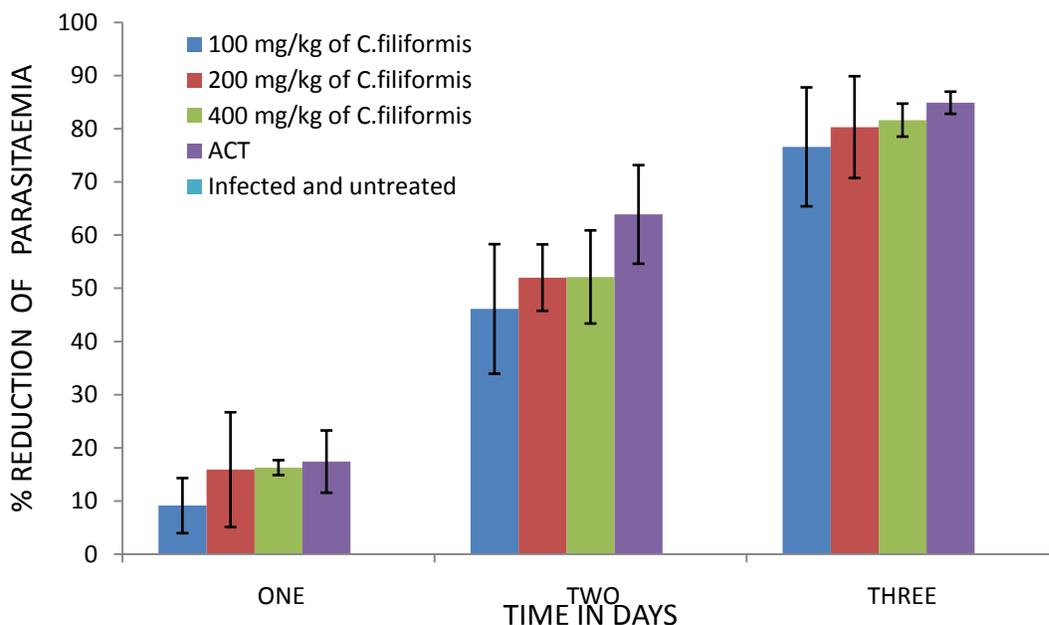


Figure 1: Effect of Treatment on Parasite Count.

Red Blood Cell Count (Rbc)

This result (figure 2) shows significant reduction (p<0.05) in the percentage red blood cell count of the infected-untreated

group when compared to the treatment groups. The uninfected-untreated group showed no significant variation (p>0.05) with the various treatment groups.

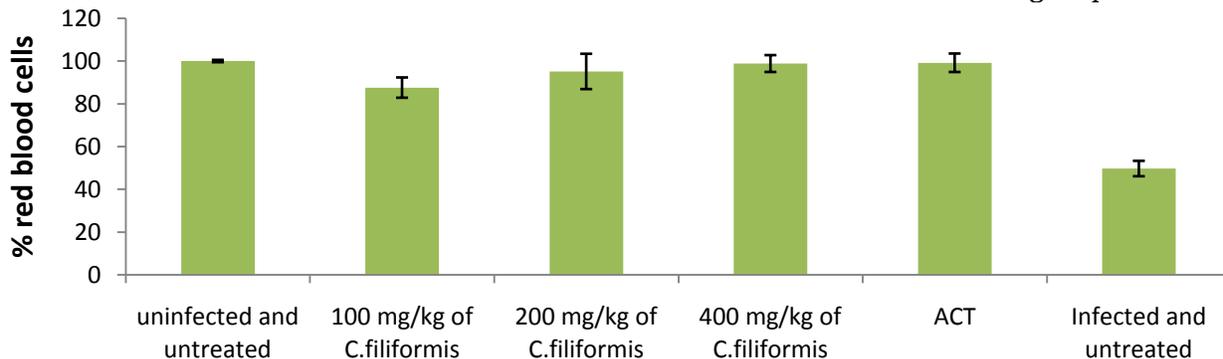


Figure 2: Effect of Treatment on Red Blood Cell Count.**Packed Cell Volume**

Figure 3 shows that the packed cell volume for the treated groups significantly increased ($p < 0.05$) when compared to the infected-untreated group, a comparison of the uninfected-

untreated group against groups 4 and 5 (400mg/kg and ACT) showed no remarkable difference in their packed cell volume while groups 2 and 3 (100mg/kg and 200mg/kg) are significantly ($p < 0.05$) lower than those of groups 4 and 5.

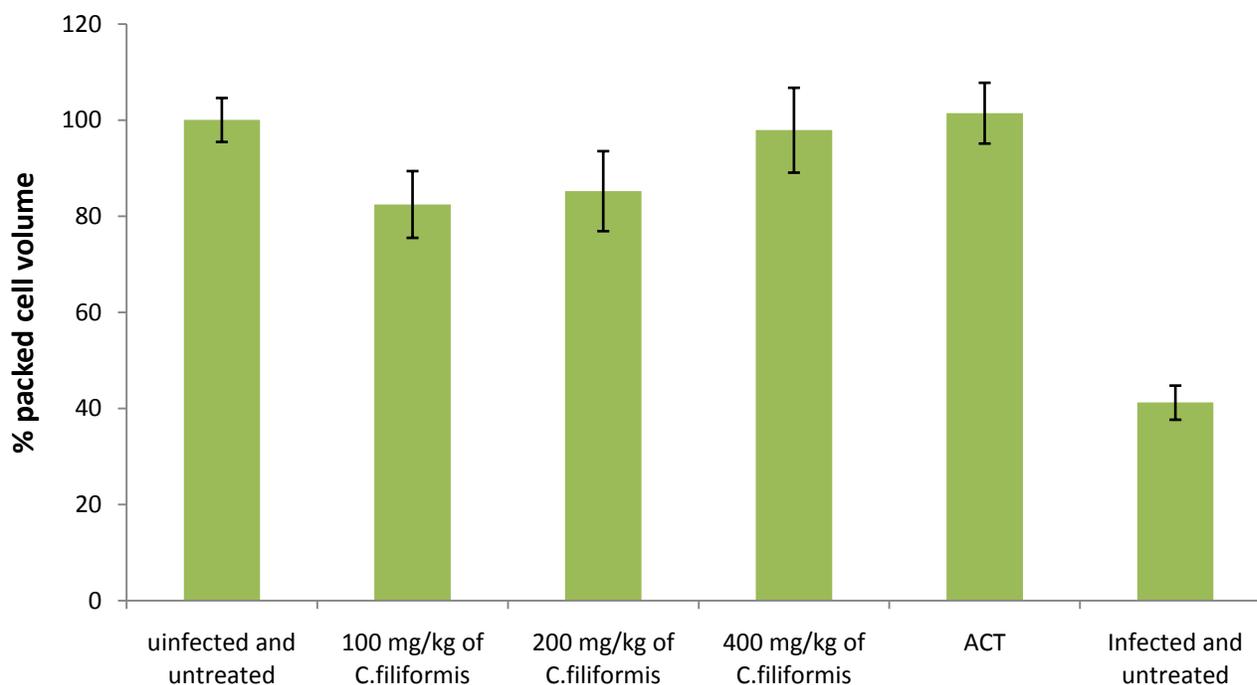
**Figure 3: Effect of Treatment on Packed Cell Volume.****Hemoglobin Concentration**

Figure 4 shows that the hemoglobin concentration increased with increasing dose of the plant extract, with no significant difference amongst the groups treated with extract, uninfected-untreated

group and the group treated with Standard drug (ACT). The hemoglobin concentration of the infected-untreated group decreased significantly ($p < 0.05$) compared to all other groups.

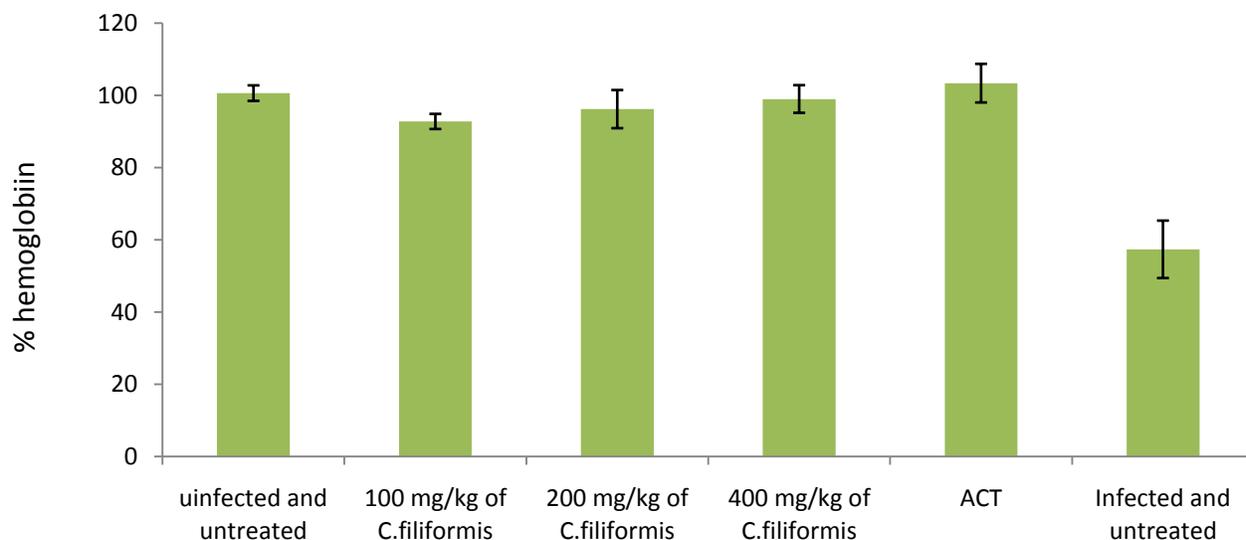


Figure 4: Effect of Treatment on Hemoglobin Concentration.

White Blood Cell Count

The WBC count recorded in this study was highest in the group treated with standard drug and lowest in the uninfected-

untreated group. Groups treated with plant extract presented WBC counts that increased with increasing extract dose (Figure 5).

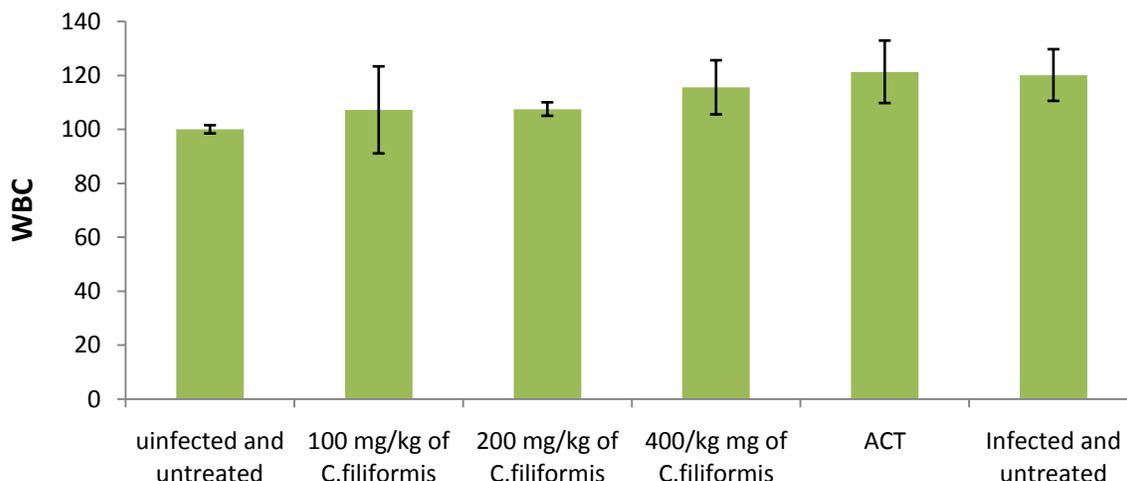


Figure 5: Effect of Treatment on White Blood Cell Count.

Biochemical Parameters

Alanine aminotransferase

The effect of treatment on serum alanine aminotransferase enzyme activity as shown in Figure 6 indicates that the percentage ALT was highest in the infected-untreated group, varying

significantly ($p < 0.05$) with those of the treatment groups as well as the uninfected-untreated group. ALT values for the groups treated with plant extracts showed no significant difference ($p > 0.05$) when compared with that of the group treated with standard drug.

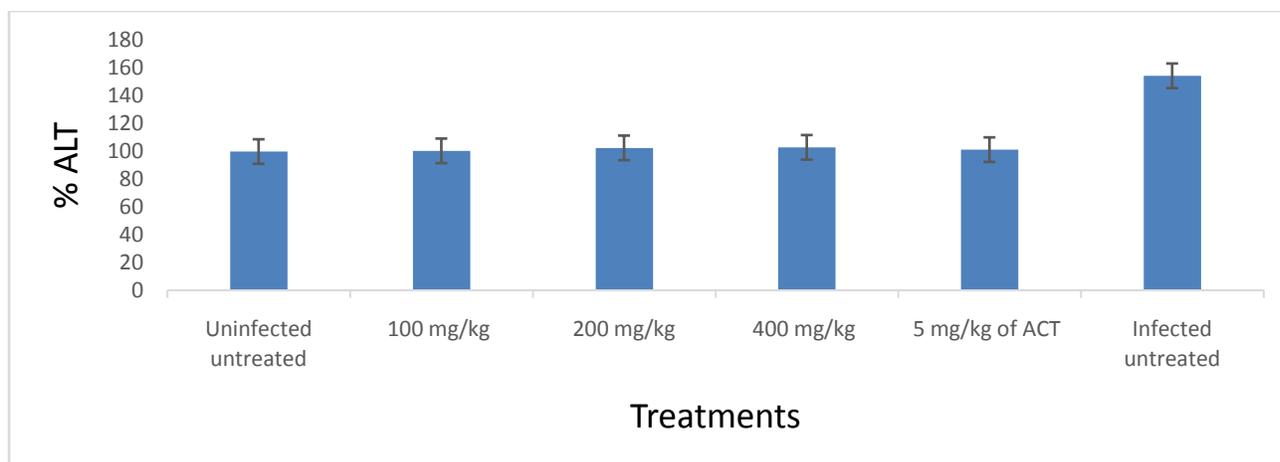


Figure 6: Effect of Treatment on Serum Alanine aminotransferase (ALT) Activity

Aspartate aminotransferase

Percentage AST activity (Figure 7) was highest in the infected-untreated group while it increased in dose dependent

manner in the groups treated with extract although not remarkably. There is no significant difference in AST levels in the different treatment groups.

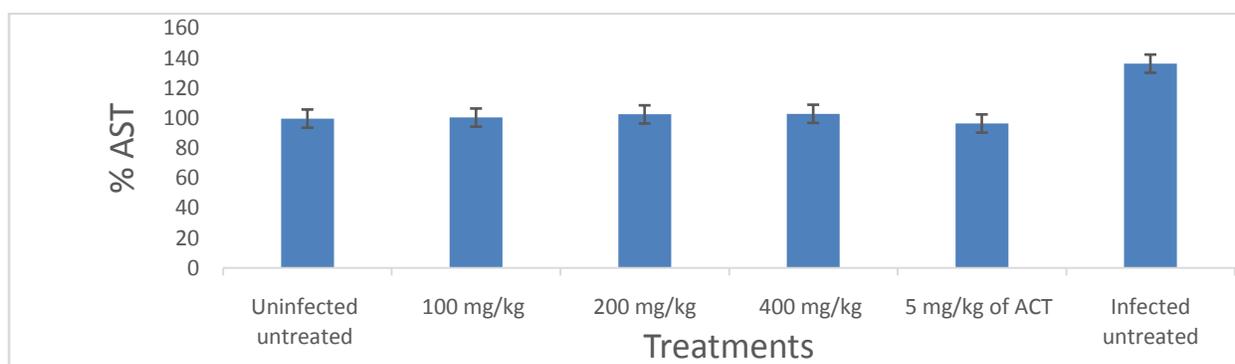


Figure 7: Effect of Treatment on Serum Aspartateaminotransferase Activity

Acute Toxicity

Screening of plant extracts for their activities against microorganisms or disease conditions is closely related to the need to ascertain their toxic potentials [22]. There was no sign of toxicity in the group administered 1600mg/kg body weight of the extract while mortality occurred in the groups treated with 2900mg/kg and 5000mg/kg body weights of the extract. Hence the mean lethal dose (LD_{50}) of Ethanolic extract of *Cassythafiliformis* was calculated to be 2154mg/kg body weight of the extract which is more than 20 times the effective minimum dose of 100mg/kg body weight

DISCUSSION

of the extract used in the study. This finding agrees with the report of [23] which stated that if the lethal dose of a substance is three times more than the minimum effective dose, the substance is considered a good candidate for further investigations. Plant extracts should not only be efficacious but safe for consumption. The extract could therefore be described as safe and could explain their safe use by indigenous people of Nigeria in management of diseases, though no literature has revealed their use for malaria treatment. This high value of LD_{50} therefore implies that ethanolic

extract of *C.filiformis* safe for use as phytomedicine.

Parasitaemia Suppression

P. berghei has been used in studying the activity of potential antiplasmodials *in vivo* in rodents [24], and it produces diseases similar to those of human plasmodial infection [25]. We observed that the parasite load in the infected-untreated group continuously increased throughout the period of the study, this supports the view point that parasitaemia increases progressively after infection or inoculation until the point of death in the absence of suitable treatment [26]. However, the extract treatment produced increased percentage reduction in parasite load as the dose concentration of the extract increased in a manner that is comparable with the percentage parasitaemia reduction produced by the standard drug. The foregoing implies that the different doses of the extract tested inhibited the malaria parasite activity to certain extents. A major drawback in the use of medicinal plants is the partial loss of activity against parasites when administered *in vivo* which may be due to lack of uptake of the extracts to physiologically active levels [27]. The antiplasmodial activity shown in (Figure 1) is in line with our previous finding that ethanolic leaf extract of *Sarcocephalus latifolius* produced antiplasmodial effect that showed direct proportionality with the dose of the extract administered, such that the higher doses produced higher parasitaemia suppression [28].

Haematological parameters

Haematological changes are some of the most common complications in malaria and they play a major role in malaria pathogenesis. These changes involve the major cell types such as RBCs, leucocytes and thrombocytes [29]. Haematological indices such as RBC count, WBC count, PCV and Hb concentration examined in this study presented certain level of alterations in *P.berghei* infected mice which corroborated the report of [30], that changes in haematological parameters are likely to be influenced by any disease condition including endemic

diseases, such as malaria, that can affect health of mankind with various clinical presentations. The pathogenesis of anaemia during malaria infection is not clearly understood. However, it is thought to result from the parasites primarily targeting the red blood cell resulting in RBCs destruction, accelerated removal of both parasitized and non-parasitized cells [31], depressed as well as ineffective erythropoiesis with dyserythropoietic changes and anemia of chronic disease [32], [33].

Other factors causative to anemia in malaria include decreased red blood cell deformability, splenic phagocytosis and/or pooling, so they have an increased rate of clearance from the circulation [34]. We noted a significant decrease ($p < 0.05$) in the RBC count of the infected-untreated group of animals when compared to the treated groups which could be attributed to RBCs destruction by the malaria parasites infection. More so, the RBC counts of the groups treated with extract and the group treated with standard drug showed no significant difference ($p > 0.05$) when compared to the uninfected-untreated group which suggests that the extract inhibited the malaria parasite and as well restored the RBC count of the infected animals. The PCV and Hb of the infected-untreated group diminished significantly ($p < 0.05$) with respect to the treated groups. The elevated levels of PCV, Hb, RBC count in the treated groups compared to the infected-untreated group suggest the extract relieved the anemic condition caused by malaria parasite infection. More so, we observed an elevated WBC count in the infected-untreated (negative control) group, suggesting the stimulation of immune system to combat the parasite, because the major functions of the white blood cell and its differentials are to fight infections, defend the body by phagocytosis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response [35].

Biochemical Parameters

Measurement of the enzymatic activities or marker enzymes in tissues plays a significant role in the diagnosis and assessment of toxicity of plant extracts [36]. Elevated levels of serum AST and ALT in the infected-untreated group observed in this study might have resulted from the Plasmodium parasite infection which caused degenerative changes in the hepatocytes and consequent release of the enzyme in the blood stream [37]. This significant increase in serum levels of selected marker enzymes is an evidence

of hepatotoxicity which may be as a result of leakage from the cells through peroxidative damage of membranes [3]. However, the AST and ALT levels were drastically lowered in the groups treated with the extract, which suggests that the extract has the ability to activate the body defense mechanism to protect the body in the event of infection. It also clearly indicates that on continued treatment, the plant extract may possibly stabilize the plasma membrane as well as help in healing of the hepatic tissue damage [7]

CONCLUSION

Antiplasmodial activity of Ethanol extract of *C. filiformis* is by this study scientifically established for the first

time, validating its folkloric use for the treatment of malaria.

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