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Antiplasmodial Activity of Methanolic Extract of *Achyranthes aspera* Shoot against *Plasmodium berghei* Infection in Albino Mice

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Abstract

Malaria remains one of the diseases that lack satisfactory treatment worldwide and continued to pose serious challenges with rapid spread of resistant parasite. Achyranthes aspera leaf, stem, and root extracts were found to exhibit antiplasmodial activities. The shoot has little or not been investigated despite its vital chemicals. Based on this, the study attempted to investigate antiplasmodial activity of methanol extract Achyranthes aspera shoot against Plasmodium berghei infection in mice. Achyranthes aspera shoot was sliced, air-dried, then pulverized into powdered form then extracted with methanol, filtered and concentrated with rotary evaporator at 40°C. The crude extract obtained was subjected to phytochemical analysis followed by acute toxicity study. Antiplasmodial effect of the extract was investigated in Swiss albino mice infected with $1x10^7$ Plasmodium berghei (NK-65) strain intrperitonealy. Eight groups of 5 mice were used; group 1: normal control, group 2: infected mice and untreated, group 3&4: infected mice + standard drugs, group 5-7: infected mice + extracts (200 mg/kg, 400 mg/kg, 600 mg/kg body wt.), group 8: infected mice + vehicle. During the experiment, parasitemia levels and PCV were monitored. Body weight and temperature were also measured.

The study found that the plant shoot contains vital phytochemicals (alkaloids, saponins, phenols and flavonoids) and is safe with LD_{50} greater than 5000 mg/kg body weight of extract of mice. Treating Plasmodium berghei infected mice with methanol extract of the plant shoot displayed remarkable effect as evidenced by the reduction in their parasitemia levels and increase survival rate in a manner comparable to chloroquine and artemisinin. The methanol extract of A. aspera is safe, possess vital phytochemicals and exhibited antiplasmodial activity in mice. As it is a good antimalarial candidate, further research should characterize the active component(s) and their mode of action.

Keywords: *Achyranthes aspera*, shoot, phytochemicals, antimalarial, *Plasmodium berghei*, Albino mice.

1. Introduction

Malaria still remains one of the diseases in which its treatment is not satisfactory worldwide and continued to pose serious challenges to people from both rural and urban communities in Nigeria and other African nations (Nguta et al., 2010). In 2019, it was estimated to have affected 219 million people, where 435,000 have died from the disease (WHO, 2018). Sub-Saharan Africa

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was reported to account for about 94 % of the malaria case out of which 25 % is from Nigeria (WHO, 2019).

The disease is mainly caused by *Plasmodium falciparum* and *P. vivax*, which have become increasingly resistant to available antimalarial drugs (Muregi et al., 2003; Qais et al., 2011). This has pushed some people to resort heavily in the use of herbal claimed medicinal plants' preparations as alternative treatment to meet their primary health care needs, to be able to overcome the enormous and persisting challenges of resistance, and adverse side effects such as cardiotoxicity associated with available antimalarial drugs (White, 2007; Qais et al., 2011). These research have been geared toward finding effective and safer agent mainly from plants since, medicinal plants have been a promising source for discovery and development of novel drugs. This have yielded a positive results in the past recent years where principle or active component in some of the recent drugs of choice against malaria infection such as artemisinin was isolated from plant known as *Artemisia annua* (Katuura et al., 2007).

Achyranthes aspera is a perennial shrub, popularly known as "pricky chaff flower" which belongs to the *Amaranthaceae* family, and a habitat of Asia, South Americ and Africa (Jain et al., 2006). In Nigeria, it is known locally as "Kiban Katangare" (Hausa), "Ndefiat" (Mwaghavwul) and "Kigye tukusan" (Ron). The plant is used by traditional medicine practitioners in the treatment of fever, particularly malaria fever, dysentery, asthma, hypertension and diabetes (Girach, Khan, 1992). Scientific studies reveal that Achyranthes aspera contained numerous active constituents with several medicinal properties. Among the important component are; ecdysterone, achyranthine, betaine, pentatriacontane, 6-pentatriacontanone, hexatriacontane and triacontane which were identified from leaves, seeds and roots parts of *Achyranthes aspera* (Tang, Eisenbrand, 1992).

The antimalarial effect of Achyranthes aspera has been studied by several researchers. The antiplasmodial studies started with the use of rodent models that have been validated through the identification of several antimalarials of Mefloquine and Artemisinin. In view of their proven use in the production of treatment outcome for human infection, thus these models remain a standard part of drug discovery and development pathway (Fidock et al., 2004). The study conducted by (Inbaneson et al., 2012) on the antiplasmodial activity of the leaf, stem, and root extracts of Achyranthes aspera in vitro reports that plants were effective against *Plasmodium falciparum* with IC_{50} values between 50 and 100 µg/ml. Following literature reports on antimalarial activity of *Achyranthes aspera* reveals that, the shoot part of the plant has little or not been investigated despite its vital chemicals and antimicrobial activity (Parvenu, 2018). Based on this, the current study attempted to investigate antiplasmodial activity of methanolic extract of *Achyranthes aspera* shoot against *Plasmodium berghei* infection in mice model.

2. Materials and methods

Plant collection and identification

Fresh *Achyranthes aspera* shoot were collected from Nchiya in Mangu Local Government Area of Plateau State, Nigeria. It was authenticated at the Herbarium Unit of the Forestry Research Institute, Jos, Plateau State, Nigeria.

Chemicals

All chemicals and solvents used were of analytical grade procured from Sigma Aldrich, USA.

Experimental Animals

A total of Thirty five Swiss albino mice of both sexes with age range of about 6 to 8 weeks were purchased from the Animal House Unit of Pharmacology Department, Faculty of Pharmaceutical Sciences, University of Jos, Plateau State Nigeria. They were kept in standard clean cages with 12/12 h light/dark photoperiod, and fed with animal's feeds (ECWA – Vital feeds, Jos) and tap water *ad-libitum*. The study was approved by the Institute of Animal Ethical Committee as regulated by the board for the purpose of control and supervision of experimental animals. Ethics approval and consent to participate was obtained with approval number (UJ/FPS/F17-00379). The mice were handled with humane care and quarantine for two weeks before used for the experiment.

Plant Extraction

The plant shoot was washed with tap water, air-dried under shade for a period of 7 days, then pulverized using mortar and pestle into powdered form, and was extracted with methanol as described by (Yared et al., 2012). Exactly, 80 g of powdered plant shoot was mixed with 300 ml of methanol and kept at room temperature (25^{o}C) for 72 hours with intermittent shaking using shaker

at 12,000 rpm. It was then filtered through a cheese cloth and concentrated using rotary evaporator at 40°C then finally air dried. Portion of the crude methanol extract obtained was put in an air-tight sterilized container and kept at 4°C in a refrigerator until used.

Phytochemical screening

Preliminary phytochemical analysis of methanolic extract of Achyranthes aspera shoot was done following method described by (Harbone, 1984).

Acute toxicity

Acute toxicity was performed according to guidelines of Organization of Economic Corporation and Development 425 (OECD, 2001). Briefly, three groups of five female mice each were weight and administered orally with 2000 mg/kg and 5000 mg/kg bwt^{-1} of mouse of the aqueous extract in a single dose using oral intubation tube. Mice were deprived of food for 3 hours prior to dosing. After each extract dose administration, observation was done at 30 min interval for 4 hours then there after 24 hours for any behavioral change or death. The animals were observed for 14 days for various sign of toxicity including hair erection, diarrhoea and mortality.

Parasites and inoculation

Chloroquine sensitive *Plasmodium berghei* (NK-65) strain obtained from National Institute of Medical Research (NIMR), University of Ibadan, Oyo State, Nigeria was used. The parasites were maintained in vivo by serial passage of blood from infected mice to non-infected ones on weekly basis. Inoculation was done by intraperitoneal injection of mice with $1x10^7$ *Plasmodium berghei* (NK-65) strain infected erythrocytes. Infection was then allowed to be established for 72 hours and observed by collecting blood from their tails and used for parasitemia determination as described by Ryley, Peters, (1970).

Experimental Animals and Grouping

A total of forty mice were used for the study which were randomly divided into eight groups of five mice each; seven groups were mice infected with *Plasmodium berhgei*, while one group is non-infected mice which served as normal control. Treatment with plant extract at three different doses lasted for about five days as follows; after the experimentation mice were not euthaniced, but the survival of each mouse recorded.

- Group 1 (Normal control): Received normal feed and distilled water only;
- Group 2 (Negative Control): Infected Mice Untreated;
- Group 3 (Standard drug control 1): Infected Mice Treated with 10 mg/kg $b.wt^{-1}$ chloroquine;
- Group 4 (standard drug control 2): Infected Mice Treated with 10 mg/kg $b.wt^{-1}$ Artemisinin (ACT);
- Group 5 Infected Mice + 200 mg/kg body $wt.^{-1}$ methanolic extract of A. aspera shoot;
- Group 6 Infected Mice + 400 mg/kg body $wt.^{-1}$ methanolic extract of A. aspera shoot;
- Group 7 Infected Mice + 600 mg/kg body $wt.^{-1}$ methanolic extract of A. aspera shoot;
- Group 8 Infected Mice + 0.2 ml of 2 % tween 80.

Determination of Parasitemia, Mean Survival Time and PCV

The standard method of (Ryley, Peters, 1970) was followed to evaluate curative activity. Standard inoculation of $1x10^7$ *P. berghei* parasitized red blood cells was injected intrapertoneally into mice on the first day. After 72 hr, mice were divided into eight groups of five mice in each group. Different doses of methanolic extract of A. aspera (200, 400 and 600 mg/kg/day) were administered orally to these groups. Chloroquine (10 mg/kg/day) and Artemisinin (25 mg/kg/day) were given to the two positive control groups and only distilled water to the negative control group. The extract and drugs were given once daily for 5 days. Parasitemia levels were monitored daily from each mice group. Blood was collected by tail rub, smeared on microscopic slides, fixed with absolute methanol and stained with 10 % giemsa for 10 mins, then rinse with water. Immersion oil was added to the stained slide where the smeared parasitized red blood cells were examined under x100 magnification using Olympus microscope (Olympus-CH). Three different fields on each slide were examined to calculate the average parasitemia (Ural et al., 2014).

Parasitemia was calculated by using the formula below:

$$\% \text{ Parasiteamia} = \frac{\text{Numberofparasitized RBC}}{\text{total numberofRBC}} x100 \quad (12).$$

The mean survival time for each group was determined by finding the average survival time (days) of mice (post-inoculation) over a period of 30 days (D0–D29) as a principle compound that prolonged survival time beyond 12 days is regarded as active (Ural et al., 2014; Mulisa et al., 2018).

The Packed Cell Volume was measured by taking blood from the mouse tail with heparinized mircro-hematocrit tubes to ¾ heights and sealed with sealing clay. The tubes were centrifuged with sealed ends outwards for 5 mins at 12,000 rpm. PCV was determined using micro-hematocrit reader (Hawskley and Sons, England) and calculated Gilmour, Skyle, 1951.

$$PCV = \frac{\text{Volme of erythrocytes in a given volume of blood}}{\text{total blood volume}}$$

Determination of the Body weight and rectal body temperature of mice: The body weights of mice were measured using sensitive weighing balance (Ohaus, USA) on day 0 before infection and day 8 in the curative test. The average body weight was compared with control group over time in each group as described by (Dikasso et al., 2001). The rectal body temperature of mice in all groups were measured using clinical digital thermometer, on day 0 before infection and days 8 in the curative test to see the effect of extract or rectal body temperature. The mean body temperature was compared with control group over time in each group.

Statistical analysis

Data from the experiment were expressed as mean ± Standard Error of Mean (SEM). Means were analyzed by one way analysis of variance (ANOVA) and compared by Duncan's multiple range test (DMRT) (Duncan, 1957). Significant difference was accepted at $P < 0.05$.

3. Results

Yield and Phytochemical Content of Methanolic extract of *Achyranthes aspera* shoot

The yield of the methanolic extract of *Achyranthes aspera* shoot is about 12.5 %. The phytochemicals identified were alkaloids, tannins, saponins, balsam, and phenols.

Acute toxicity of the Methanolic extract of Achyranthes aspera Shoot

No mortality was recorded during the toxicity study neither was there any adverse effect in the behavior of mice administered both extract doses. The LD_{50}value was therefore considered to be more than 5000 mg/kg body weight for oral administration of methanolic extract of Achyranthes aspera Shoot in mice.

Effects of Methanolic extract of Achyranthes aspera shoot on PCV of Infected Mice

The result of PCV of mice infected with *P. berghei* following treatment with methanolic extract of Achyranthes aspera shoot is shown in Figure 1. There was a fluctuation in PCV levels of untreated mice and those treated with the plant extract and standard drugs particularly at day 6 of the experiment but were still within normal value. Packed cell volume of normal control mice increased gradually through-out the experimental period.

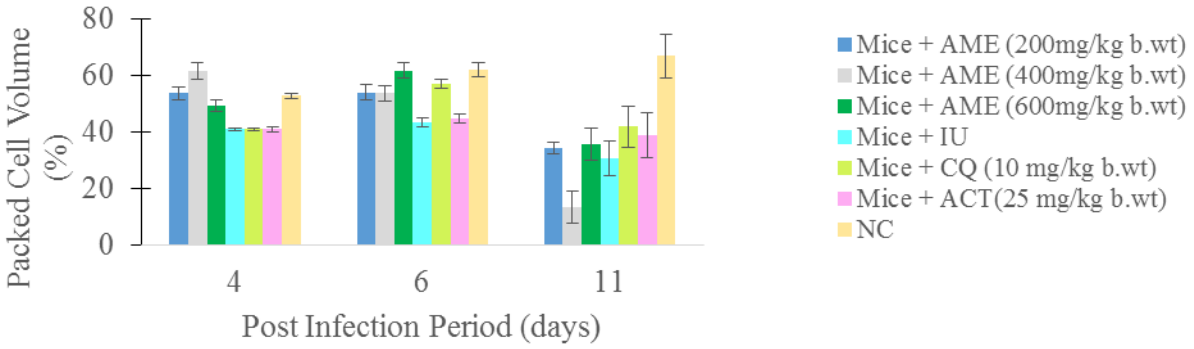

Fig. 1. Change in Packed Cell Volume of Mice Infected with P. berghei following Treatment with Methanolic Extract of Achyranthes aspera Shoot

Mice + AME = Infected mice treated with Achyranthes aspera shoot methanol extract, Mice + IU = Infected mice untreated, Mice + CQ = Infected mice treated with Chloroquine (10 mg/kg), Mice + ACT= Infected mice treated with Artimisinin (25 mg/kg).

Effect of Methanolic extract of *Achyranthes aspera* Shoot on Temperature of Infected Mice

The change in temperature of mice infected with *P. berghei* following treatment with methanolic extract of *Achyranthes aspera* shoot is presented in Figure 2. The study showed no significant changes in temperature of infected mice treated when compared to the untreated and normal control mice.

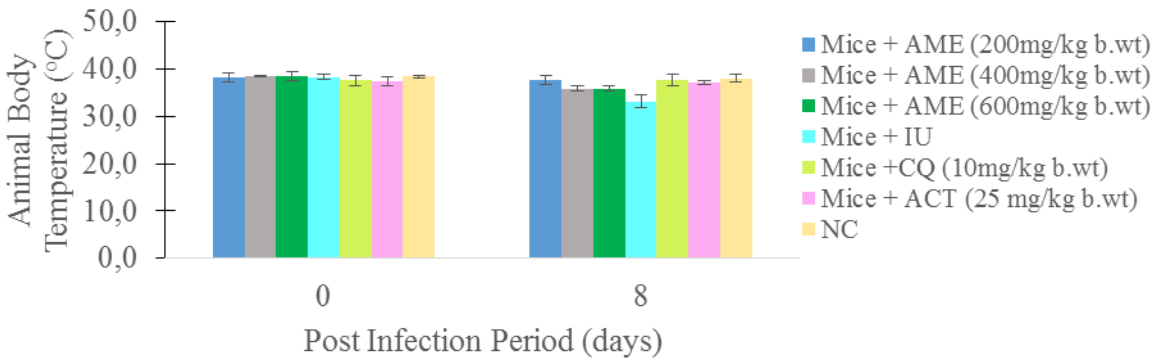

Fig. 2. Change in Body Temperature of Mice Infected with P. berghei following Treatment with Methanolic Extract of Achyranthes aspera Shoot

Mice + AME = Infected mice treated with Achyranthes aspera shoot methanol extract, Mice + IU = Infected mice untreated, Mice + CQ = Infected mice treated with Chloroquine (10 mg/kg), Mice + ACT= Infected mice treated with Artimisinin (25 mg/kg)

Effect of Methanolic extract of Achyranthes aspera Shoot on Weights of Infected Mice

The result of body weights of mice infected with *P. berghei* following Treatment with methanolic extract of Achyranthes aspera Shoot is shown in Figure 3. There was gradual increased in weight by infected mice following treatment with plant extract as was in the normal control. But, slight loss in body weight was recorded by mice administered the standard drugs compared to those not treated.

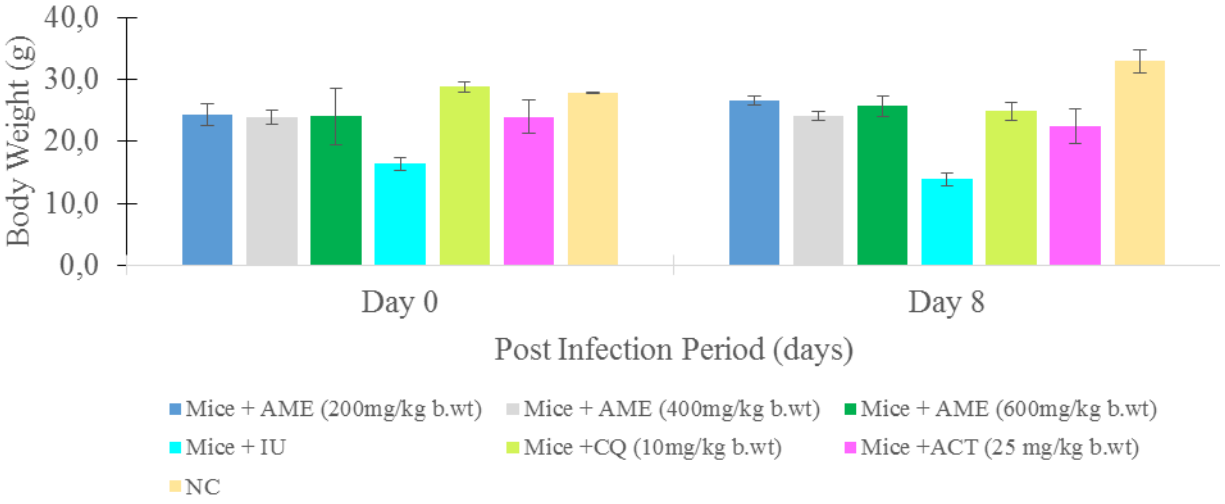

Fig. 3. Change in Body Weight of Mice Infected with P. berghei following Treatment with Methanolic Extract of Achyranthes aspera Shoot

Mice + AME = Infected mice treated with Achyranthes aspera shoot methanol extract, Mice + IU = Infected mice untreated, Mice + CQ = Infected mice treated with Chloroquine (10 mg/kg), Mice + ACT= Infected mice treated with Artimisinin (25 mg/kg).

Antiplasmodial Effect of Methanolic extract of Achyranthes aspera Shoot in Infected Mice

The antiplasmodial activity of methanolic extract of *Achyranthes aspera* is presented in Figure 4. The result showed a gradual reduction in parasitemia levels in mice treated with methanol extract, Chloroquine and Artimisinin compared with the untreated mice. The reduction of parasitemia by methanol extract of the plant shoot was in a dose dependent manner where higher reduction in parasitemia was recorded in mice that were administered 600 mg/kg body wt. plant shoot extract.

The result of mean survival rate of infected mice is presented in Figure 5. The study recorded high survival rate of mice treated with Chloroquine followed by those that received Artimisinin and 600 mg/kg dose of the methanol extract of *Achyranthes aspera* shoot.

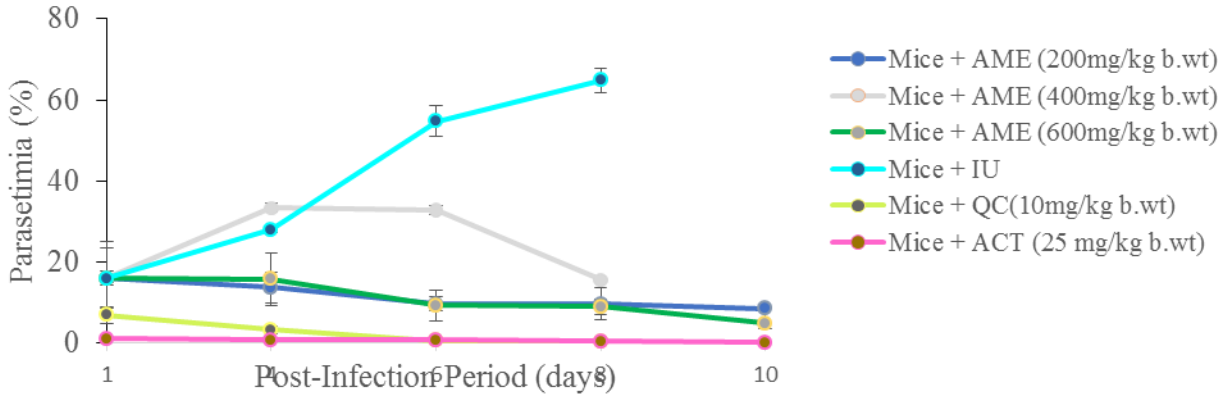

Fig. 4. Change in Parasitemia Levels in Mice Infected with Plamodium berhei following Treatment with Methanolic Extract of Achyranthes aspera Shoot

Mice + AME = Infected mice treated with Achyranthes aspera shoot methanol extract, Mice + IU = Infected mice untreated, Mice + CQ = Infected mice treated with Chloroquine (10 mg/kg), Mice + ACT= Infected mice treated with Artimisinin (25 mg/kg).

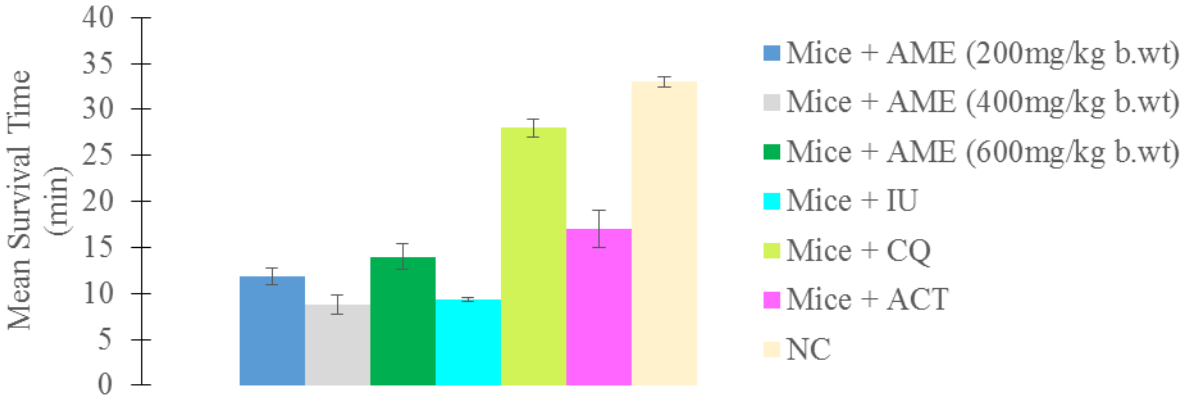

Fig. 5. Survival Rate of Mice Infected with *Plasmodium berghei* following Treatment with Methanolic Extract of *Achyranthes aspera* Shoot

Mice + AME = Infected mice treated with *Achyranthes aspera* shoot methanol extract, Mice + IU = Infected mice untreated, Mice + CQ = Infected mice treated with Chloroquine (10 mg/kg), Mice + ACT= Infected mice treated with Artemisinin (25 mg/kg).

4. Discussion

In this study, methanolic extract of the shoot part of the plant contain vital phytochemicals like alkaloids, saponins, phenols and flavonoid. The alkaloids, phenols and saponins identified in methanolic extracts of *Achyranthes aspera* shoot in the study are in-line with the findings reported in a studies conducted by Sharma, Chandhary (2015) and Tiwari et al. (2018) on methanolic and ethanolic leaf extract of *Achyranthes aspera*. Alkaloids were reported to exert antimalarial activity by blocking protein synthesis in *P. falciparum* (Liu et al., 2017), likewise phenols and tannins are reported to exhibit antimalarial activities (Soh et al., 2012). Phenols have been reported in several plants as the major pharmacological contributors, thereby suggesting that same chemical compounds might be likely the key player for the vast medicinal potentials of *Achyranthes aspera*. Saponins are other important type of bioactive chemical constituents which are involved in plant disease resistance due to their anti-microbial activity, which found to be associated with vast biological activities (Anyasor et al., 2010).

The methanolic extract of *A. aspera* shoot was studied for in vivo antiplasmodial activity in order to ensure its safety and efficacy in various doses according to (Chandel, Bagai, 2010). Mice infected with parsasite die within 7-10 days of infection in normal cause of infection in the present study, treating *Plasmodium berghei* infected mice with methanolic extract, showed a significant effect as evidence by the reduction in their parasitemia level and increased in survival rate in manner comparable to chloroquine and artemisnin treated drugs. This may be due to the phytochemicals identified and the reduced pathologic effects of the extract. The anitmalarial activity observed may be due to these constituents, the methanolic extract is highly effective and activity could be due to synergistic effect of these secondary metabolites. In vitro studies revealed that the methanolic extract of *Uvariopsis congolana* leaves (*Amaranthaceae*) displayed good activity (4.57±076 µ/ml against *P. falciparum* (W_2) strain in vitro (Hilou et al., 2006).

The decrease in PCV levels after the administration of the extract could be due to the loss or destruction of red blood cell. This may be explained by the fact that, since, the *Plasmodium* parasite is localized in the cells and treatment may likely involve lysing cells which after clearing the parasites, the cells gradually divide and replenish resulting in PCV fluctuation. Similar scenario have been reported in a studies conducted by Yang et al., 2005 with *Dodonae angustipholia*, Bantie et al. (2014) with *Croton macrostachyus* extracts, chloroform leaf extract and diethyl ether leaf extracts of *Eucalyptus cameldulensis* (Ishaya et al., 2019a; Ishaya et al., 2019b). Changes in body weight and rectal temperature of infected mice observed in the study are associated with general features of malaria disease (Langhorne et al., 2017). However, treating malaria with antimalarial agents may reverse the changed, extract prevented weight loss at all dose associated with *P. berghei* infection, and is an indication of ameliorative potentials of the plant extract on the aneamia induced by the malaria infection. This could also be due to the presence of appetite enhancing and immune modulatory component(s) in the extract (Fenthahun et al., 2017). The insignificant changes observed in the rectal temperature could be as a result of increase in tropical metabolite rate thus indicating potential active components(s) responsible for this effect were likely found in a good concentration that have antimalarial activities in the extract.

5. Conclusion

The study confirmed methanolic extract of *Achyranthes aspera* shoot, to possess vital phytochemicals. Extract is safe as no mortality was reported and possessed good antiplasmodial activity. Further research to explore the active compound and mode of action in recommended.

- Conflict of Interest

The authors declare that they have no competing interest

- Acknowledgements

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- Ethics and consent approval to participate:

The study was approved by the Institute of Animal Ethical Committee, University of Jos. Ethical clearance obtained with approval number (UJ/FPS/F17-00379).

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