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Assessment of Phytochemical Constituents of Stem Bark Extract of *Albizia adianthifolia* and its *in-vivo* Antiplasmodial Activity on Mice infested with *Plasmodium berghei*

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ABSTRACT: Malaria, one of the most debilitating diseases, has a huge impact on large populations in tropical and subtropical areas. *Albizia adianthifolia* is a perennial evergreen that thrives in moist tropical forest areas. This study intended to assess the phytochemical constituents of stem bark extract of *Albizia adianthifolia* and its *in-vivo* antiplasmodial activity on mice infested with *Plasmodium berghei*. The plant's antimalarial effects at 100, 200 and 400 mg/kg b. wt. dosages against the chloroquine-sensitive strain, *Plasmodium berghei* were examined using the 4-day suppressive and rane's, tests. In this experiment, 25 mice were classed into five groups (3 treatment and 2 control groups, each with five mice). Parasitaemia, existence time, body mass, and packed cell volume were used to assess the antiplasmodial activity of the extracts. In the 4-day suppression investigation, all doses of the crude extract significantly ($P < 0.05$) reduced parasitemia, compared to the negative control, with the strongest suppressive effect of 59.93% obtained at dose 400 mg/kg b. wt. Similarly, the Rane's test revealed significant ($P <0.05$) curative effect (69.03%) at the highest dose, when compared to the untreated control. According to the findings of the phytochemical screening, the extract contains alkaloids, flavonoids, anthraquinones, tannins, triterpenes, sterols, and saponins. Also, treatment of *P. berghei*-infected mice with the crude extract in both suppressive and curative tests considerably extended the mean survival time relative to the negative control, notably at the highest dose (400 mg/ kg b. wt.). Thus, this study shows that *Albizia adianthifolia* stem bark extract possesses antiplasmodial efficacy against *P. berghei*-infected mice, which may be due to its phyto-constituents.

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Keywords: *Albizia adianthifolia; Antiplasmodial; Plasmodium berghei*; Parasitaemia; Phytochemicals

The perennial, evergreen shrub *Albizia adianthifolia* (Schumach) is widespread in Africa and Central South America. The work of Maroyi (2018) showed that *A. adianthifolia* contains triterpenoids and saponins in roots while the leaves contain steroids, alkaloids, tannins, flavonoids, and saponins. However, cardiac glycosides and anthraquinones were absent from the leaves. Literature reveals that *A. adianthifolia* has been used in various folk remedies such as skin conditions, respiratory tract infections, headaches, sinusitis, bronchitis, inflamed eyes, tapeworm, malaria, as well as analgesic, diabetes, purgative, anti-inflammatory, reproductive issues in women, sexually transferred infections, and as a psychotic principle (Maroyi, 2018; Abubakar and Majinda, 2016). The bark and leaves of *A. adianthifolia* have been applied in traditional African medicine to improve memory and treat neurodegenerative disorders like Alzheimer's disease and memory loss (Maroyi, 2018; Abubakar and Majinda, 2016). Malaria is among the most pressing public health problems in developing countries, principally in Africa. The disease, which affects more than 90 countries and sickens and kills half a million people annually, causes significant morbidity and mortality in tropical areas and impedes development in the world's poorest countries (WHO, 2021; Okoro, 2020a). The majority of the malaria load is still carried by the African Region. In 2020, the Region was home to 90% of all malaria cases (228 million cases) and 96% of all malaria deaths (602, 000 deaths) worldwide. Moreover, children under the age of five accounted for 80% of all malaria-related deaths in the territory. Between 2000 and 2019, the mortality rate (deaths per 100, 000 persons at risk) declined from

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149.6 to 56, while the case occurrence of malaria (cases per 1000 people at danger) decreased from 368 to 222.9 (WHO, 2021). The bulk (55%) of malaria occurrences worldwide in 2020 were found in Nigeria (26.7%), the Democratic Republic of the Congo (12.0%), Uganda (5.4%), Mozambique (4.2%), Angola (3.4%), and Burkina Faso (3.4%). Over 50% of malaria deaths globally were caused by four countries: Nigeria (31.9%), the Democratic Republic of the Congo (13.2%), the United Republic of Tanzania (4.1%), and Mozambique (3.8%). Death rates increased from 5% to 25% in eight countries: Angola, the Democratic Republic of the Congo, Guinea-Bissau, Liberia, Namibia, Nigeria, South Sudan, and Uganda (WHO, 2021). Plasmodium falciparum, the most hazardous malaria parasite, is accountable for over 50% of all cases of malaria. Cerebral malaria, severe anemia commonly accompanied with splenomegaly, headache, hepatomegaly, and hemoglobinuria with renal failure are among the basic effects (Okoro, 2020a). Common clinical signs of malaria include fever, chills, nausea, and flu-like symptoms. In earlier studies, typical malaria sequelae included hematological and biochemical alterations (Djouwoug *et al*., 2021, Okoro *et al.,* 2016). The occurrence of insecticide-resistant mosquitoes, the rapid expansion of multi-drug resistant Plasmodium parasites, and the want of effective immunizations are some of the challenges in controlling and ultimately eradicating malaria. In reality, attention has been drawn more to plant-based natural remedies since the discovery of quinine and, more recently, artemisinin derivatives (Okoro *et al.,* 2016). The need for novel, efficient malaria therapies is highlighted by the evolution of resistance to previously effective malaria drugs, such as Artemisinin-based combination therapy (Okoro, 2019). In the bulk of developing countries, herbal treatments have long been the main sources of medicine. A seemingly endless reservoir of chemicals for the management of the multisystemic malaria sickness has long been made available by the use of medicinal herbs (Djouwoug *et al*., 2021). Therefore, the objective of this work is to assess the phytochemical constituents of stem bark extract of *Albizia adianthifolia* and its *in-vivo* antiplasmodial activity on mice infested with *Plasmodium berghei*.

MATERIALS AND METHODS

Drug and Chemicals: Chemicals and drug used had analytical quality, procured majorly from Sigma Chemical Co., USA.

Plant Collection and Extraction: Dr. A.H. Akinnibosun, Department of Plant Biology/Biotechnology, University of Benin, Nigeria, authenticated the plant material, *Albizia adianthifolia* (Voucher Number: UBH-A502), which was taken from a bush at Abraka, Delta State. The plant's bark was shade-dried, crushed, and immersed for three days in 80% methanol with periodic shaking. It was then filtered with Whatman No. 1 filter paper, concentrated using a rotary evaporator, and freeze-dried.

Phytochemical Screening: Following established protocols (Trease, and Evans, 1989; Harborne, 1973), the plant extract was examined for the presence of secondary metabolites such as alkaloids, polyphenols, cardiac glycosides, flavonoids, anthraquinones, coumarins, phlobatannins, tannins, triterpenes, steroids, and saponins.

Animals and Parasites: Throughout the experiment, male White Swiss albino mice weighing 22–30 g and aged 5–6 weeks were used. The mice were procured from the animal house of the Faculty of Basic Medical Sciences, Department of Anatomy, Delta State University, Abraka, and were fed food pellets and water as needed throughout the study. The Faculty of Science Research and Ethics Committee of Delta State University, Abraka, gave its clearance for the research (REL/FOS/ 22/03), which were carried out in compliance with internationally acknowledged standards for the use and care of laboratory animals (National Institutes of Health, 2011). The antimalarial experiment was conducted using a *Plasmodium berghei* ANKA strain that is chloroquine sensitive. In the following weeks, serial blood transfusions between infected and uninfected mice were used to maintain the parasites in the laboratory.

Acute Oral Toxicity: The primary test was carried out for an examination of the extract's acute oral toxicity to ascertain the 50% lethal dosage (LD_{50}). This was done using the two-phased method as described earlier (Okoro *et al.,* 2015) to determine the lethal and safe doses for the extracts.

Antiplasmodial Activity (In Vivo)

Parasite Inoculation: As previously indicated (Peters, 1975), blood smear preparation allowed for the assessment of donor mice's parasitemia. Donor mice were employed with parasitemia levels of 30–40% that had previously been exposed to *P. berghei*. Blood was extracted into heparinized vacutainers containing trisodium citrate (0.5%) after animals were killed while under anesthesia. The blood was diluted with saline (0.9%) based on the parasitemia levels. Healthy mice were intraperitoneally injected with 0.2 mL of the inoculum, which contained 1×10^7 parasitized erythrocytes, for the antiplasmodial experiment.

Animal Grouping: Twenty five mice were randomly divided into five groups (n= 5): three treatment groups and two control groups as follows:
Group I: Mice + 10 mL / kg b. wt. of distilled water (negative control).
Group II: Mice + 10 mg /Kg b. wt. of artesunate (positive control).
Group III: Mice + 100 mg/kg b. wt. of extract (orally)
Group IV: Mice + 200 mg/kg b. wt. of extract (orally)
Group V: Mice + 400 mg/kg b. wt. of extract (orally)

Four-Day Suppressive Test: This test was done in accordance with Peters' (1975) method to see if the extract could treat schizontosis in the early stages of illness. Five groups of 25 male infected mice were randomly assigned (5 mice per group). Treatment started two hours after the infection. The test drugs were given every day up until the third day (D3). Blood was taken from each mouse's tails on the fifth day (D4), and thin blood films were made to determine the percentage of suppression and the parasitemia levels. Day 0 (before infection) and Day 4, the packed cell volume (PCV) and weight measurements of each mouse were made.

Rane's (Curative) Test: The therapeutic potential of the crude extract was evaluated using the Belay *et al.* (2018) methodology. A 0.2 mL inoculum containing 1×10^7 parasitized RBCs was administered intraperitoneally to the mice on the first day (day 0). After 72 hours of the infection (day 3), the mice were split into five groups (n = 5). The treatment groups received 100, 200, and 400 mg /kg b. wt. of extract, whereas the negative and positive control groups received 10 mL/kg b. wt. of distilled water and 10 mg kg b. wt. of artesunate respectively for four consecutive days, (days 3-days 6), during which the parasitaemia levels were checked on days 3 and 7. Each mouse's packed cell volume (PCV), and body weight were assessed before and after the initial dosing (D3) and (D7).

Data Analysis: Data are presented as mean± SD. Comparisons were made by one-way analysis of variance (ANOVA), and multiple comparisons between tests were made using the Tukey's (post hoc) test. The outcomes were deemed statistically significant at $p < 0.05$. For all studies, GraphPad Prism 6 for Windows was used (GraphPad Software, Inc.).

RESULTS AND DISCUSSION

The result of primary phytochemical assessment of the stem bark extract of *A. adianthifolia* is presented in Table 1. According to the results of the phytochemical screening, the extract contains alkaloids, flavonoids, coumarins, anthraquinones, tannins, triterpenes, steroids, and saponins. Phytochemicals, or secondary plant metabolites, have a variety of pharmacological and physiological effects on living beings (Okoro, 2020b; Sultana *et al.*, 2009).

Table 1. Phytochemical composition of *A. adianthifolia* bark extract.

Tests	Results
Alkaloids	+
Anthraquinones	+
Cardiac glycosides	-
Coumarins	+
Flavonoids	+
Phlobatannins	-
Saponins	+
Steroids	+
Tannins	+
Triterpenes	+

(-): Absent; (+): Present

The acute oral toxicity test showed that the methanolic stem bark extract of *A. adianthifolia* was non-toxic after extract administration at doses up to 5000 mg/kg b. wt. Thus, the doses used in this experiment are therefore typically safe. *In vivo* models rather than *in vitro* studies are often used in antimalarial research to account for a potential prodrug effect and the immune system's role in the eradication of infection (Hilou *et al.*, 2006). The parasite, *P. berghei* was selected for this experiment due to its better accessibility and because it is a suitable parasite (Fidock *et al.*, 2004). It was determined in this study, whether the crude extract of the stem bark of *A. adianthifolia* had antiplasmodial properties using conventional models. A preliminary 4-day suppression test was performed to evaluate the extract's schizonticidal activity.

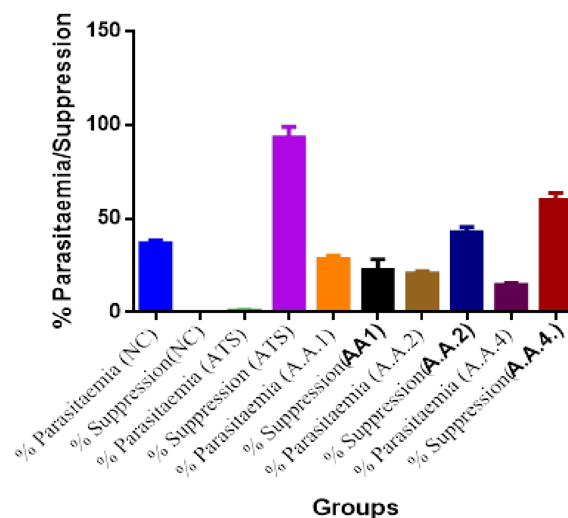

Fig 1. Effect of *A. adianthifolia* extracts on the % parasitaemia level of *P. berghei*-infected mice in the suppressive test. *values are presented as mean± SD (n=5). **NC- negative control, ATS-artesunate (positive control), A.A.1- 100 mg/kg of *A. adianthifolia* extract, A.A.2- 200 mg/kg of *A. adianthifolia* extract, A.A.4- 400 mg/kg of *A. adianthifolia* extract.

The administration of extract to infected mice led to a significant ($p < 0.05$) reduction in parasitaemia as contrasted in a dose-dependent fashion to the malarial control group (A.A.4 > A.A.2 > A.A.1). The percentage of parasitaemia that was suppressed the most (59.93%) was obtained in the group that got the highest dose of plant extract (400 mg/kg b. wt.).

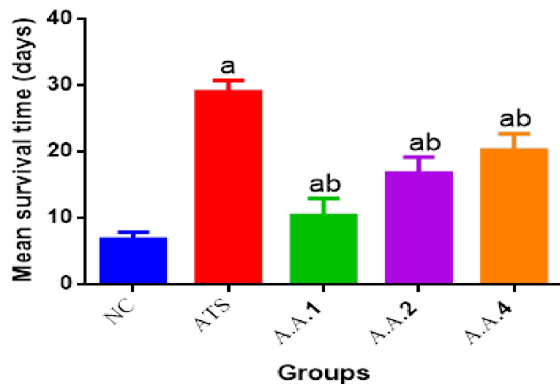

Fig 2. Effect of *A. adianthifolia* extract on the average survival time of *P. berghei*-infected mice (suppressive test). *values are presented as mean± SD (n=5). **NC- negative control, ATS-artesunate (positive control), A.A.1- 100 mg/kg of *A. adianthifolia* extract, A.A.2- 200 mg/kg of *A. adianthifolia* extract, A.A.4- 400 mg/kg of *A. adianthifolia* extract. *** a= compares with NC ($p < 0.05$), b= compares with artesunate control ($p < 0.05$).

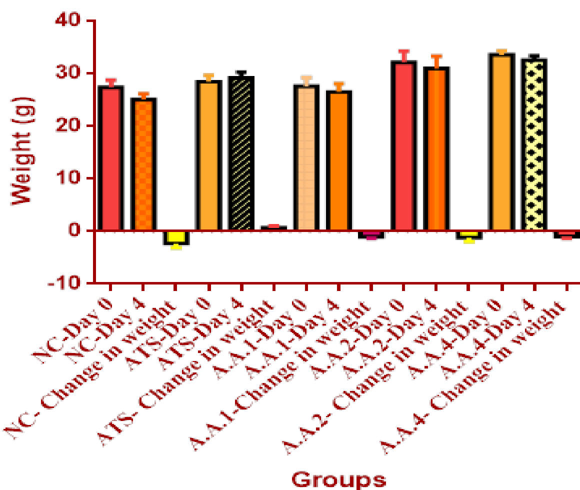

Fig 3. Effect of *A. adianthifolia* extracts on the body weight of *P. berghei*-infected mice (suppressive test). *values are presented as mean± SD (n=5). **NC- negative control, ATS-artesunate (positive control), A.A.1- 100 mg/kg of *A. adianthifolia* extract, A.A.2- 200 mg/kg of *A. adianthifolia* extract, A.A.4- 400 mg/kg of *A. adianthifolia* extract.

The average survival periods of *P. berghei*-infected mice were significantly extended at all dosages, but the 400 mg/kg dose extract in particular led to a lengthy survival duration of 20.20±0.84 days. However, effect of the positive control was more significant than the extract-treated groups (Figures 1 and 2). The administration of the extract to mice checked a major ($p < 0.05$) decline in PCV and weight loss in the animals that received 200 and 400 mg/ kg of extract, when contrasted to the malaria control group, but no difference was observed in the change in PCV between the malaria control animals and the A.A.1 extract treated group . Also, treatment with the standard drug precluded a decline ($p < 0.05$) in both PCV and body weight after the 4-day treatment when compared with the negative control group (Figures 3 and 4).

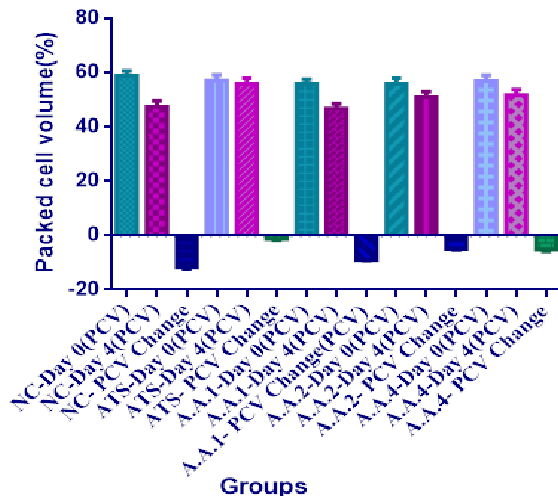

Fig 4. Effect of *A. adianthifolia* extract on the packed cell volume level of *P. berghei*-infected mice (suppressive test). *values are presented as mean± SD (n=5). **NC- negative control, ATS-artesunate (positive control), A.A.1- 100 mg/kg of *A. adianthifolia* extract, A.A.2- 200 mg/kg of *A. adianthifolia* extract, A.A.4- 400 mg/kg of *A. adianthifolia* extract.

When therapeutic capability of *A. adianthifolia* extract was assessed in the curative test, a dose dependent decrease ($p < 0.05$) in parasitaemia level on days 7 compared to days 3 of the experimentation was noticed. Similar to the suppressive test, the maximum suppression of parasitaemia (69.03%) was attained on day 7 with the dose of 400 mg/kg b. wt. of extract. This observation may be due to the fact that natural products often contain active compounds at low concentrations, where activity may not be observed at lower doses (Batista *et al*., 2009). *In vivo* antimalarial activity is categorized as moderate, good, or very good if the extract demonstrates a parasitemia suppression percentage of at least 50% (Tarkang *et al*., 2014). However, the artesunate control group had a far greater level of parasitaemia suppression on day 7, at 97.33% (Figure 5). This might be as a result of the extract's phytochemicals not having accumulated to the point where they would have caused a significant suppression at low doses or because the extract acts more slowly than artesunate does. But according to Adugna (2014), a chemical is deemed to be active when parasitemia is reduced by 30% or more. Therefore, it can be inferred that, the extract of *A.*

adianthifolia has antimalarial properties and the results supports its antimalarial effects as earlier mentioned (Abubakar and Majinda, 2016). The mean survival time is a crucial metric used to assess the antimalarial efficiency of plant extracts (Fidock *et al*., 2004). In this analysis, the average survival time was higher in the group of animals who received the maximum dose of extract (21.20±1.30 days) and the standard drug (28.80±0.84 days) (Figure 6). This lends more evidence to the idea that *P. berghei* was inhibited, which lessens its overall pathologic impact on the research mice. In both experiments, mice given doses of the plant's extract had a significantly shorter average survival time ($P < 0.05$) than animals treated with the standard drug; this difference could be explained by the extracts' quick clearance or decreased potency. If a plant compound can prolong the survival of infected laboratory animals compared to the negative control, it is deemed to be effective against malaria. A main chemical that extends survival time past 12 days is said to be active (Ural *et al*., 2014). Thus, the results found from the study support the antiplasmodial effects of the plant's extract.

A decrease in packed cell volume is one trait of malaria-infected mice. The extract's effectiveness at preventing the hemolysis that the rising parasitemia level caused was evaluated in this experiment using PCV (Okokon *et al.*, 2006). The impacts of increasing parasitemia include erythropoietic inhibition, dyserythropoiesis, removal and/or ruin of infested RBCs, and removal of healthy RBCs (Asagba *et al.,* 2010a; Lamikanra *et al.*, 2007). In this experiment, treatment of *P. berghei*- infested animals with the extract averted a considerable ($p < 0.05$) shortfall in body mass and PCV, particularly in the groups A.A. 2 and A.A.4 mice when contrasted with the malaria control group. Similarly, the dispensation of artesunate to *P. berghei*-infected mice precluded a significant ($p < 0.05$) decrease in the body mass and PCV of the animals relative to the malaria control group (Figures 7 & 8). Thereby further supporting the antiplasmodial actions of the extract. Anemia is one of the recognized signs of malaria, which happens when red blood cells are destroyed. It is thus expected that potent antimalarial drugs will be able to reverse or stop the parasite-caused anemia (Awoke and Arota, 2019). The antimalarial activity of plants has been connected to diverse active compounds, like flavonoids, alkaloids, saponins and steroids. The antimalarial effect of flavonoids has been linked to reduction of hemoglobin breakdown by preventing the act of cysteine protease enzyme falcipain-2 (Hidayati *et al*., 2020; Asagba *et al.,* 2010b). The saponins also have an antimalarial impact by causing the erythrocyte cells to lyse and rupturing the cell membranes of infected erythrocytes (Ungogo *et al*., 2020). By precluding the assembly of parasite DNA and RNA, alkaloids have been proven to reduce parasitemia levels (Talapko *et al*., 2019). Numerous investigations have found that steroids and their derivatives have antimalarial effects, however the mechanism is still unknown (Uzor, 2020). According to Krieg *et al*. (2017), arylmethylamino steroids are particularly efficient antiplasmodial components due to their capacity to stop transmission, oral availability, and low level of toxicity.

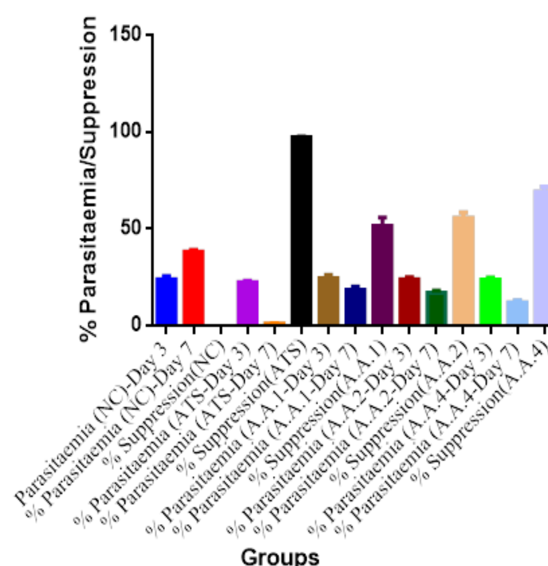

Fig 5. Effect of *A. adianthifolia* extract on the % parasitaemia level of *P. berghei*-infected mice in the curative test
*values are presented as mean± SD (n=5). **NC- negative control, ATS-artesunate (positive control), A.A.1- 100 mg/kg of *A. adianthifolia* extract, A.A.2- 200 mg/kg of *A. adianthifolia* extract, A.A.4- 400 mg/kg of *A. adianthifolia* extract.

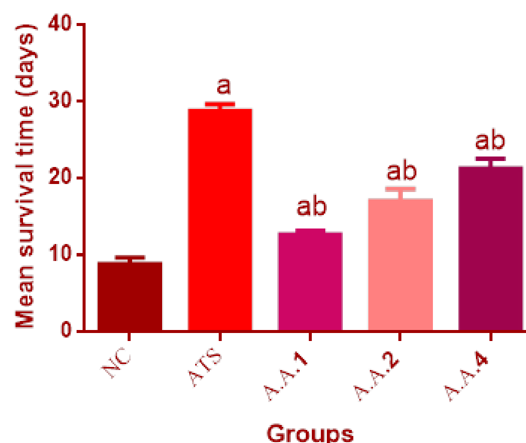

Fig 6. Influence of *A. adianthifolia* extract on average survival time of *P. berghei*- infested mice (curative test).
*values are presented as mean± SD (n=5). **NC- negative control, ATS-artesunate (positive control), A.A.1- 100 mg/kg of *A. adianthifolia* extract, A.A.2- 200 mg/kg of *A. adianthifolia* extract, A.A.4- 400 mg/kg of *A. adianthifolia* extract. . *** a= compares with NC ($p < 0.05$), b= compares with artesunate control ($p < 0.05$).

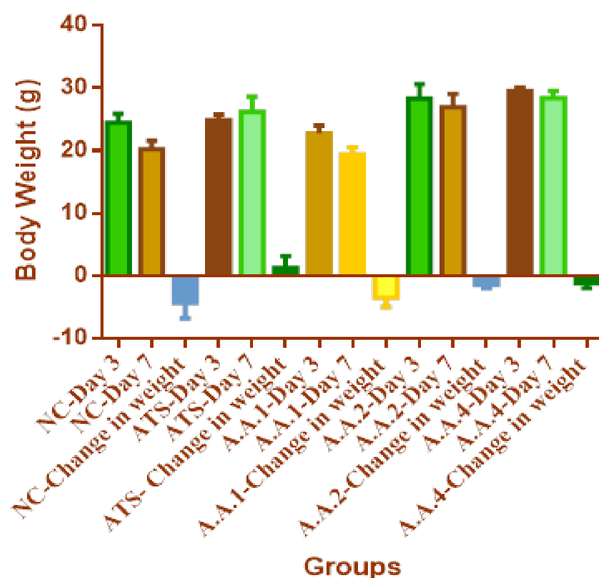

Fig 7. Influence of *A. adianthifolia* extract on body mass of *P. berghei*- infested mice (curative test).
*values are presented as mean± SD (n=5). **NC- negative control, ATS-artesunate (positive control), A.A.1- 100 mg/kg of *A. adianthifolia* extract, A.A.2- 200 mg/kg of *A. adianthifolia* extract, A.A.4- 400 mg/kg of *A. adianthifolia* extract.

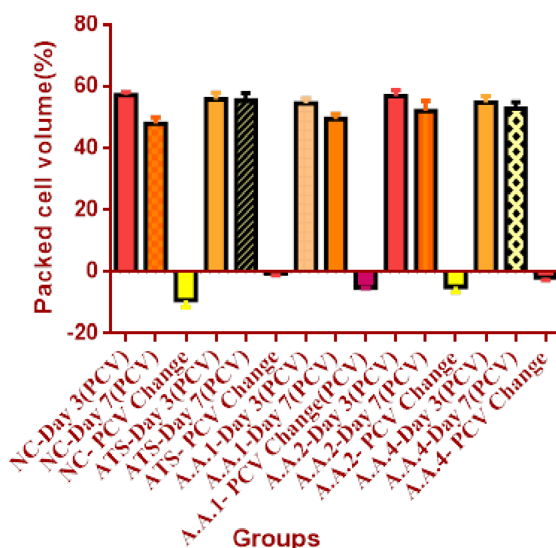

Fig 8. Influence of *A. adianthifolia* extract on packed cell volume (PCV) level of *P. berghei*- infested mice (curative test).
*values are presented as mean± SD (n=5). **NC- negative control, ATS-artesunate (positive control), A.A.1- 100 mg/kg of *A. adianthifolia* extract, A.A.2- 200 mg/kg of *A. adianthifolia* extract, A.A.4- 400 mg/kg of *A. adianthifolia* extract.

Lipophilic steroid carriers are added to this antiparasitic to boost its efficiency because they facilitate intracellular transport channels and cellular uptake. Estratriene, a steroid derivative that acts as an antiplasmodial drug, boosts cellular oxidation (Krieg *et al*., 2017). The mechanism of *A. adianthifolia* extract's action is still unknown. However, studies based on published research have shown that specific plants and seeds exhibit antiplasmodial activity by either oxidizing red blood cells/ by controlling protein synthesis, depending on their phytochemical contents (Baragana, 2015). Therefore, the metabolites present in the plant may have played a role in the antiplasmodial action produced by *A. adianthifolia* extract in this experiment.

Conclusion: Based the results of this experiment, the plant's extract is pretty safe for mice and had a moderate antimalarial effect, with the utmost dose (400 mg/kg) displaying a reasonably good reduction in parasitemia. In contrast to the malarial control, the PCV was protected and the average survival duration was increased by drastically lowering parasitemia. These findings suggest that more chemical investigation is necessary to investigate the potential for this plant to produce active antiplasmodial metabolites.

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