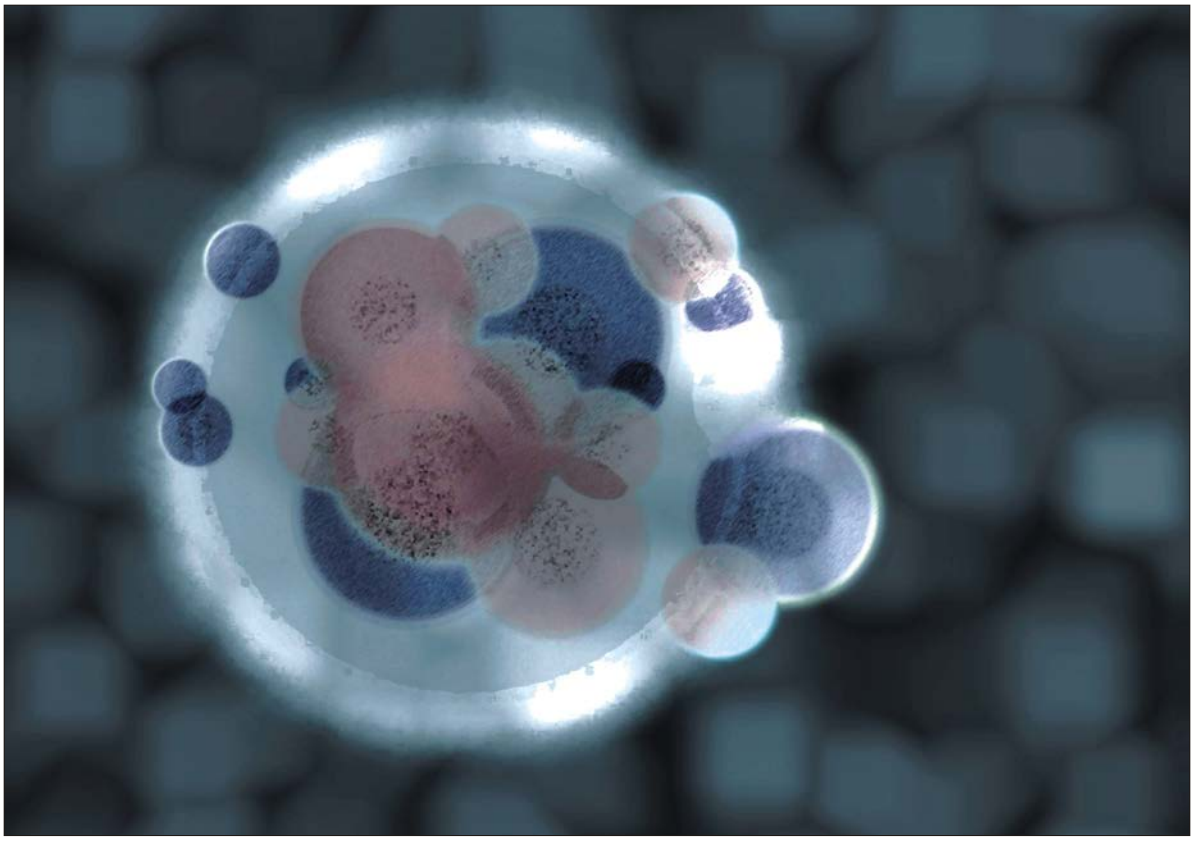

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Therapeutic Potentials of *Adansonia digitata* (Bombacaceae) Stem Bark in *Plasmodium berghei*-Infected Mice

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ABSTRACT
Malaria is one of the world's most deadly diseases which affect primarily poor populations in tropical and subtropical areas. Effort to developing more potent antimalarial from plant sources is on the increase due to the increased incidence of resistance of malaria parasites to chemotherapy. This study evaluated the therapeutic effects of aqueous and methanolic extract of *Adansonia digitata* stem bark against established infection in chloroquine sensitive *Plasmodium berghei* infected mice. Two different doses (200 and 400 mg kg^{-1} b.wt.) of aqueous and methanolic stem bark extract of *Adansonia digitata* were administered orally to albino mice. 5 mg kg^{-1} b.wt. of chloroquine was used as positive control while the negative control mice received only the vehicle (5% v/v tween 80). The extracts in the two doses exerted significant ($p<0.05$) dose dependent chemosuppressive effect at different levels of the infection treated. The results obtained showed that the 400 mg kg^{-1} b.wt. were more effective with respect to the parasite clearance than the 200 mg kg^{-1} b.wt. in the two extracts. The 400 mg kg^{-1} b.wt. of methanolic extract exhibited the highest chemosuppression. However, chloroquine at 5 mg kg^{-1} b.wt. was significantly ($p<0.05$) higher than the extract treated group. There was an increase in Packed Cell Volume (PCV) in all the extract treated groups when compared to the control and chloroquine treated group was found to be significantly higher. Also, significant mean survival time was recorded in the extract treated group compared to the control during established infection. This study showed that *Adansonia digitata* has antimalarial property which can be explored for the management of malaria.

Key words: *Adansonia digitata*, antimalaria, *Plasmodium berghei*, chemosuppression, chloroquine

INTRODUCTION

Malaria is a major prevalent disease with high mortality rate in many tropical and subtropical countries. The burden of this disease increases owing to the increasing resistance of *Plasmodium falciparum* against the commonly available antimalarial drugs.

According to the WHO, there is an estimated 225 million malaria cases, with 800,000 deaths among the 3 billion people at risk globally. About 91% of overall deaths occur in Africa with pregnant women and children under 5 years being the most affected group (WHO., 2011).

Medicinal plants are commonly used in treating and preventing specific ailments and diseases and are generally considered to play a beneficial role in healthcare. According to WHO, approximately 80% of the developing world's population meets their primary healthcare needs through traditional medicine. Synthetic drugs contain at least one

active ingredient derived from plant material. Some are made from plant extracts while others are synthesized to mimic a natural plant compound. Within the last few decades, many plants have been screened for their biological and pharmacological properties. These efforts are continually being taken to examine the merits of traditional medicine in the light of modern science with a view aimed at adopting effectively beneficial medical practice and discouraging harmful ones (Sofowora, 1986).

A variety of herbs and herbal extracts contain different phytochemical with biological activity that can be therapeutically valuable. Much of the protective effect of fruits, leaves and vegetables has been attributed by phytochemical which are the non-nutrient plant compounds. Different phytochemical have been found to possess a wide range of activities which may help in protection against chronic diseases.

Adansonia digitata, is a perennial plant, commonly called baobab, dead-rat tree, monkey-bread tree, lemonade tree in English and "Oshe" in Yoruba language. It is the most widespread of the *Adansonia* species on the African continent, found in the hot, dry savannahs of Sub-Saharan Africa.

Different parts of *Adansonia digitata* have been reported to posses medicinal properties. Leaf infusions are used as treatment for kidney and bladder diseases, blood clearing and asthma diarrhea, fever, inflammation (Van Wyk and Gericke, 2000). The fruit pulp is conventionally used against small pox measles, diarrhea, scurvy, cough and dysentery. The bark has been sold commercially in Europe for the treatment of fever, particularly that caused by malaria (Brendler *et al*., 2003).

Earlier studies by Ajaiyeoba (2005) and Musila *et al*. (2013) suggested that *Adansonia digitata* has significant antimalarial properties. However, few reports exist in the literature on the antimalarial activity of methanolic stem bark extracts of *A. digitata*.

A number of scientific studies have been performed on the plant such as on its anti-diarrheic properties (Tal-Dia *et al*., 1996), its anti-inflammatory, analgesic (pain killing) and antipyretic (temperature reducing) properties (Ramadan *et al*., 1994), its effect against sickle cell anemia (Adesanya *et al*., 1988) and its antimicrobial and antifungal activities (Le Grand, 1989). Studies on the prebiotic effect (ability to stimulate the growth and/or the metabolic activity of beneficial organisms) of the fruit pulp were performed by the University of Piacenza (Milza, 2002).

Here, we report the therapeutic activity of aqueous and methanolic stem bark extracts of *Adansonia digitata* in *Plasmodium berghei* infected mice.

MATERIALS AND METHODS

Plant material: The stem of *Adansonia digitata* (Bombacaceae) was collected from Ido-Ekiti, Ekiti State Nigeria. The plant was identified and authenticated by Mr. K.A. Adeniyi and Mr. L.T. Soyewo in the herbarium unit of Forest Research Institute of Nigeria (FRIN) with identification number (No. FHI 109806).

Extraction of plant material: The stem bark peels were air-dried at room temperature to avoid possible degradation or denaturation of their putative compounds. The air-dried stem bark of *Adansonia digitata* was blended to powder using an electric blender. This was stored in a glass container. Blended air-dried stem bark was soaked in sufficient volume of methanol for 72 h at room temperature. It was continually stirred after each 24 h. After 72 h, the mixture was then filtered and the filtrate was concentrated using rotary evaporator at 40°C. The concentrate was heated over a water bath to obtain a solvent free extract which was stored in a refrigerator at 4°C.

Experimental animals: Forty eight albino mice weighing between 18-20 g were obtained from the animal house, Institute of Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Nigeria. The animals were acclimatized for two weeks in the animal house and fed *ad libitum* on rat chow and water throughout the period of the experiment.

Parasites: The *Plasmodium berghei* was obtained from the Institute of Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Nigeria. A standard inoculum of 1×107 of parasitized erythrocytes from a donor mouse in volumes of 0.2 mL was used to infect the experimental animals intra-peritoneally.

Transfection and treatment: Estimation of the therapeutic effects of the aqueous and methanolic extract of *Adansonia digitata* stem bark on established infection was carried out according to the method described by Ryley and Peters (1970). The mice were transfected intraperitonially with an inoculums size of 1×10^7 of chloroquine sensitive strain of *Plasmodium berghei* infected erythrocytes. Parasitemia was confirmed after 72 h. The animals were divided into six groups of eight mice each and they were treated for five days, 72 h after transfection using two different concentrations (200 and 400 mg kg^{-1} b.wt. day^{-1}). The chloroquine group received 5 mg kg^{-1} b.wt. day^{-1} while the negative control received the vehicle (5% v/v tween 80) only. Blood samples were collected from the mice tails each day and thin films were made. The thin film was first fixed in 100% methanol and air-dried prior to staining with Giemsa stain. Percentage parasitemia and percentage clearance/chemosuppression were estimated.

$$\text{Parasitemia (\%)} = \frac{\text{Total number of parasitized cells}}{\text{Total number of cell}} \times 100$$

$$\text{Clearance / chemo supression (\%)} = \frac{\text{Negative control parasitemia} - \text{Parasitamia with drug}}{\text{Negative control parasitemia}}$$

Statistical analysis: Results were expressed as Mean±Standard error of mean. The Duncan multiple range test and student t-test were used to analyze and compared the results at 95% confidence level. Values of $p<0.05$ were considered significant.

RESULTS

There was a daily increase in parasitemia levels in the negative control group (Fig. 1) and significant reduction in the extract treated groups was observed. Reduction in the parasitemia level at 400 mg kg^{-1} b.wt. day^{-1} was significantly ($p<0.05$) higher than 200 mg kg^{-1} b.wt. day^{-1}.

Aqueous extract at 400 mg kg^{-1} b.wt. had (76.14±0.06) chemosuppression while the methanolic extract had (90.18±0.04) chemosuppression on the fifth day of treatment. The stem bark extract of *Adansonia digitata* produced significant ($p<0.05$) dose dependent reduction in parasitemia levels in the two doses relative to negative control group. Chloroquine showed highest chemosuppression/clearance and zero parasitemia was observed on the third day of treatment which was maintained throughout the study (Fig. 2).

In Fig. 3, the Packed Cell Volume (PCV) in the negative control was significantly lowered (20.00±0.00). In the extract treated groups, the PCV improves in a dose dependent manner. Methanolic extract at 400 mg kg^{-1} b.wt. was found to be higher (35.00±0.00) than the aqueous extract at 400 mg kg^{-1} b.wt. (30.00±0.00) after the fifth day treatment. Chloroquine treated group had the highest value (44.67±2.60) when compared with all the other extract treated groups.

A significant ($p<0.05$) increase in mean survival time was recorded in all the extract treated groups when compared to negative control. Aqueous extract at 400 mg kg^{-1} b.wt. had mean survival time of (10.9) days while methanolic extract at 400 mg kg^{-1} b.wt. had mean survival time of (15.8) days. Chloroquine treated group had the highest mean survival time of 21 days (Fig. 4).

DISCUSSION

Presently, there is no single drug that is potent against malaria infection and effective combination therapy includes artemisinin derivatives (Fidock *et al*., 2004) or mixtures of the older drugs such as combination malarone (Taylor and White, 2004; Winter *et al*., 2006). The use of artemisinin combination therapy is however limited due to its high cost and accessibility. Plants are usually considered to be possible candidates as alternative and rich source of new drugs. Majority of the population in many tropical countries depend on traditional medical remedies using herbs (Zirihi *et al*., 2005). The trends of parasitemia among the extracts of *Adansonia digitata* stem bark treatment groups and negative control appeared to demonstrate the antimalarial potential of the plant. Parasitemia in the negative control was higher than all the treatment groups. Parasitemia in the

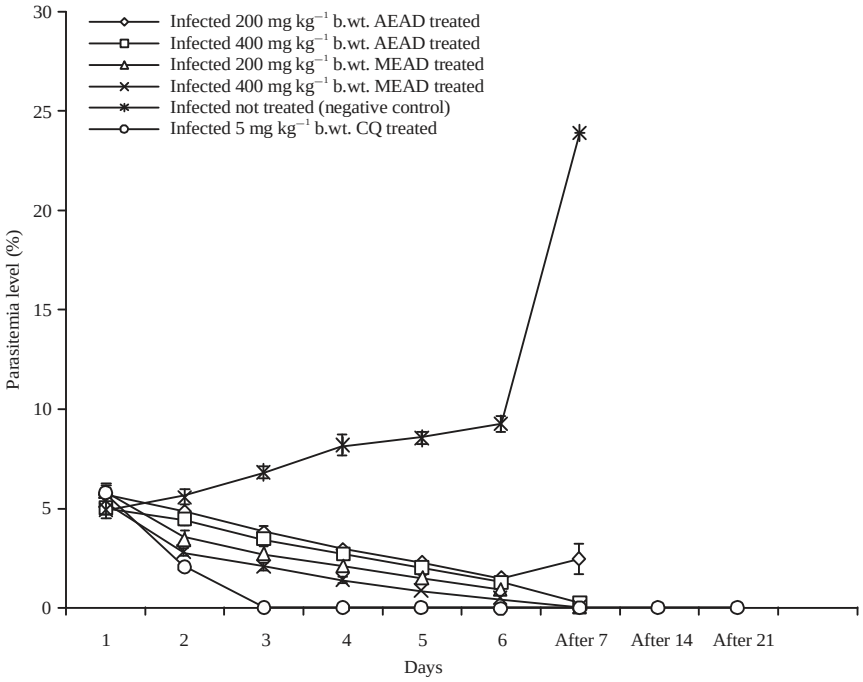

Fig. 1: Percentage parasitemia in *Plasmodium berghei* infected mice treated with extract of *Adansonia digitata* stem bark, results are expressed as mean of 8 determinations±Standard Error of Mean (SEM)

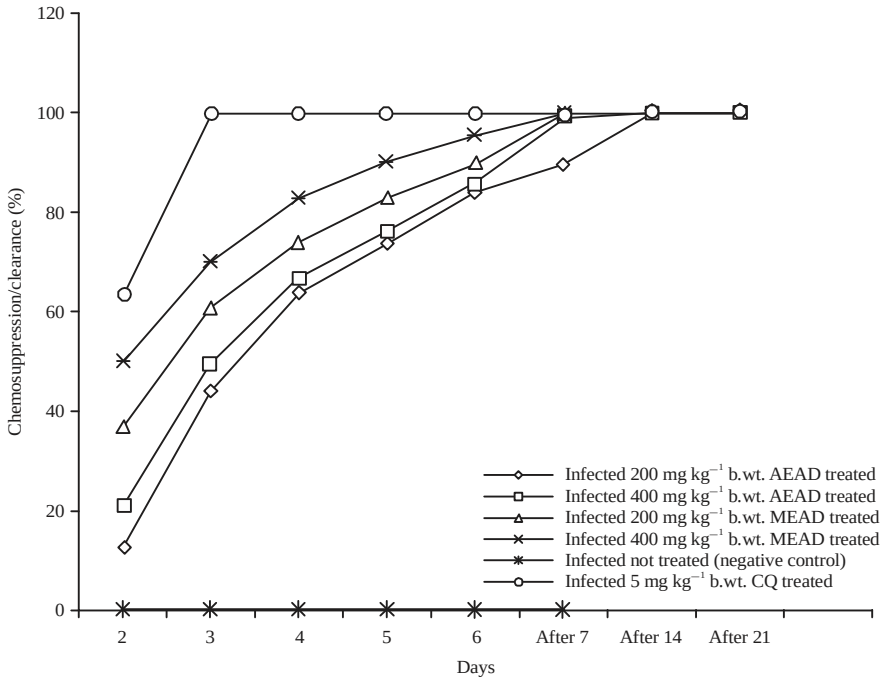

Fig. 2: Percentage clearance/chemosuppression in *Plasmodium berghei* infected mice treated with extract of *Adansonia digitata* stem bark, results are expressed as mean of 8 determinations±Standard Error of Mean (SEM)

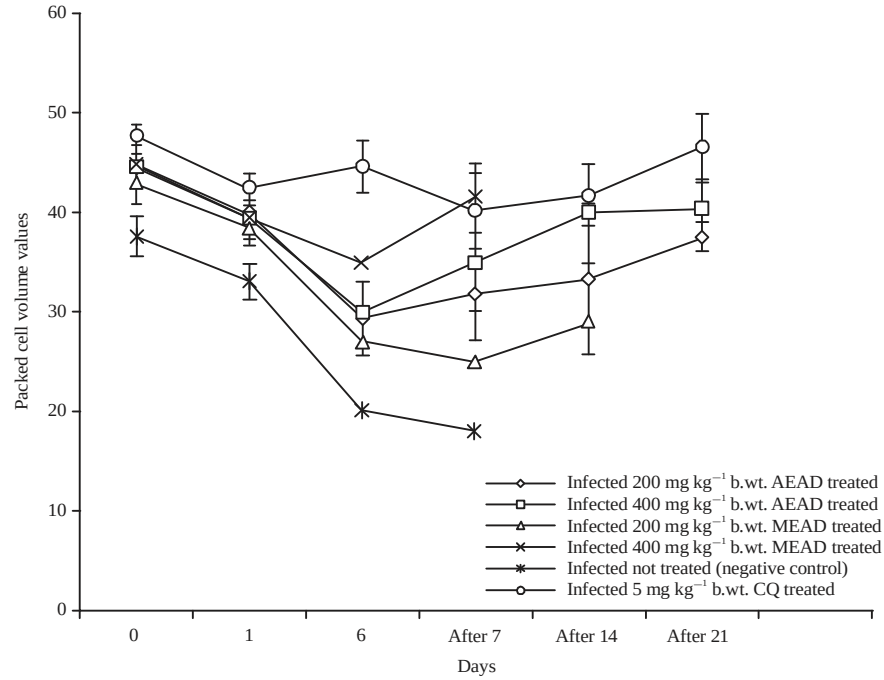

Fig. 3: Packed cell volume of *Plasmodium berghei* infected mice treated with extract of *Adansonia digitata* stem bark, results are expressed as mean of 8 determinations±Standard Error of Mean (SEM)

negative control was higher than all the treatment groups. This showed that all the treatment had effect on the growth of *Plasmodium berghei* parasites in mice. Results showed a significant ($p<0.05$) dose dependent increase in percentage chemosuppression/clearance and a significant decrease in percentage parasitemia of the extracts at the two doses. From the study, methanolic extract of *Adansonia digitata* stem bark exhibited the highest antimalaria (Fidock *et al*., 2004) activity.

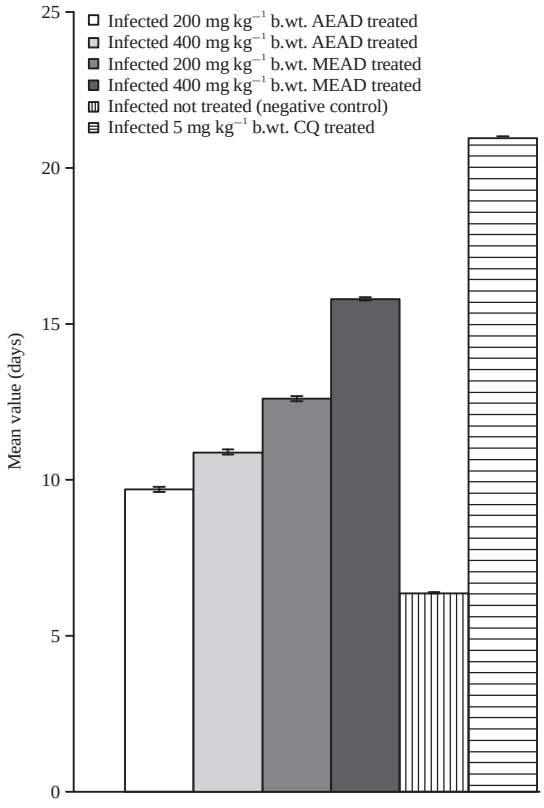

Fig. 4: Mean survival time of *Plasmodium berghei* infected mice treated with extract of *Adansonia digitata* stem bark, results are expressed as mean of 8 determinations±Standard Error of Mean (SEM)

This observation agrees with the earlier work by Ajaiyeoba (2005) and Musila *et al*. (2013), who reported that methanolic extract of the stem bark of *Adansonia digitata* were able to reduce the number of *Plasmodium berghei* parasites in mice. The significant ($p<0.05$) activity of methanolic extract of *Adansonia digitata* stem bark at 400 mg kg^{-1} b.wt. observed during established infection, though lowered was comparable to the standard drug chloroquine (5 mg kg^{-1} b.wt.). The observed antimalarial activity is consistent with the traditional use of the plant as a herbal medication against the disease in Nigeria. The lower activity of the extract could be as a result of the crude nature of the extract which can be improved by further purification. The observed higher efficacy of the standard drug chloroquine which was higher than the extract treated groups may in part be due to non selectivity of the extract or slow absorption and poor bioavailability of the extract. Although, the mechanism of action of this extract has not yet been elucidated, suggested mechanisms of action for some antimalarial compounds isolated from plants include intercalation with the parasite DNA (Kirby *et al*., 1995), inhibition of hemozoin polymerization in the parasite (Banzouzi *et al*., 2004; Onyeibor *et al*., 2005; Karou *et al*., 2007a, b), inhibition of *Plasmodium falciparum* lactate dehydrogenase (pfLDH), an essential enzyme for energy generation within the parasite through glycolysis (Royer *et al*., 1986; Gomez *et al*., 1997), inhibition of protein synthesis (Kirby *et al*., 1989), interference with the formation of mitotic spindle and the assembly of microtubules into typical axonemes in gametes, thus inhibiting the formation of mobile microgametes (Jones *et al*., 1994; Billker *et al*., 2002), enhancing elevation of red blood cell oxidation (Etkin, 1997), or inhibition of proteolytic processing of circumsporozoite protein by a parasite-derived cysteine protease, thereby preventing sporozoite invasion of host cells (Coppi *et al*., 2006). The extract could have elicited its action through either of these mechanisms or by some other unknown mechanism.

Also, a direct proportionality between parasite clearance and packed cell volume increase was observed. *Plasmodium* parasite is well known to cause red blood cell haemolysis resulting in anaemic state. There was a dose dependent increase in the packed cell volume of all the treatment groups. The low packed cell volume in the negative control is an indication of hemolytic anaemia.

A previous work by Ramadan *et al*. (1994) reported that aqueous extract of the fruit pulp of *Adansonia digitata* had a LD_{50}>8000 µg mL^{-1} which was categorized as non toxic to mice. The non-toxicity of the fruit pulp of *Adansonia digitata* explains why most of the plant parts: Fruit pulps, leaves and seeds are consumed by many communities (Kamatou *et al*., 2011; Nguta *et al*., 2011). The observed antimalarial potential in the extract treated group may be attributed to the presence of various secondary metabolites. Previous studies have also shown the antimalarial activity of alkaloids and flavonoids in plants (Okokon *et al*., 2005; Balogun *et al*., 2009). The presence of the various chemical compounds in high concentration in the extracts may be responsible for their high antimalarial potential. There was significant ($p<0.05$) increase in the mean survival time in extract treated group when compared to the control. The chloroquine treated group had the highest Mean Survival Time (MST) as there was no death recorded.

CONCLUSION

The stem bark extract of *Adansonia digitata* has therapeutic effects on *Plasmodium berghei* infected mice at higher concentration. The results of this study showed that methanolic extract possess the highest activity suggesting the presence of certain bioactive compounds in it. Based on the findings of this study, it is evident that *Adansonia digitata* possess promising and potent antimalarial effect which justifies its usage in folk medicine for the management of malaria.

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