



Antiplasmodial and immunomodulatory activities of dichloromethane extract of *Stachytarpheta jamaicensis* in *Plasmodium berghei* infected mice

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Abstract

Increased resistance of malaria parasite to first line antimalarial drugs has led to the search for alternatives in the management of malaria. This study evaluated the anti-plasmodial and immunomodulatory effect of dichloromethane plant extract of *Stachytarpheta jamaicensis* in *Plasmodium berghei* infected mice. Chloroquine-resistant *Plasmodium berghei* infected mice were separated into five treatment groups (200 mg/Kg, 400 mg/Kg, and 600 mg/Kg) with a negative control (given distilled water only) and a positive control (treated with Piperaquine-Dihydro-artemisinin), a standard drug. The plant extracts and the standard drugs were administered orally. Parasitological examinations and survival rates of the animals were monitored for 30 days, post infection. Phytochemical screening of the plant extract was performed using the standard method. Enzyme-linked immunosorbent assay was carried out to ascertain the immunomodulatory potential of the plant extract. Leaf extract of *S. jamaicensis* revealed the presence of alkaloids only. The extract showed significant ($P < 0.05$) antiplasmodial effect on Day 11 post-infection and at 200 mg/kg dose mediated parasite clearance and improved survival throughout the study. The intense and sustained TGF- β level stimulated by *S. jamaicensis* at 200 mg/kg dose may have translated into the prolonged survival observed in the mice. *S. jamaicensis* modulated both pro and anti-inflammatory responses in early and late infections, thus preventing pathological outcomes associated with excessive expression of the pro-inflammatory (IFN- γ) and anti-inflammatory (TGF- β) cytokines. *S. jamaicensis* may therefore be explored as alternatives for development of novel antimalarial and immunomodulatory candidates.

Keywords Chemosuppression · Anti-malarial · Dichloromethane · Post-infection

Introduction

Malaria is one of the most important public health problems in terms of morbidity and mortality especially in sub Saharan Africa, Nigeria inclusive causing an estimated 68 million cases and 194, 000 deaths every year. Nigeria has the highest burden of malaria globally, accounting for 27% of the global malaria burden. Malaria mostly affects pregnant mothers and children below five years of age. Recently malaria parasite has proven resistant to synthetic drugs like chloroquine leading to the search of alternative and

complementary therapy. While anti-parasite agents used in the treatment of infectious diseases prevent rapid multiplication and hamper vital physiological activities of the parasite, parasite elimination is mediated by hosts' immune system and immune modulation is crucial in combating infection (Feng et al. 2012). In fact parasites weaken immune system as a way to establish themselves in the host, it can be speculated that activation of host immune system could be an effective way to successfully combat various infectious diseases (Arif et al. 2007). Innate immunity is essential in the production of protective immune response. Artavanis et al. (2003) reported that production of pro-inflammatory cytokines which partake in both protection and pathogenesis of malaria infection is essential. It was reported by Malaguarnera and Musumeci (2002) that cytokines such as IFN- γ released in the course of infection determine the outcome of the disease. On the other hand, severe malaria

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has been associated with over production of cytokines such as TNF- α and IFN- γ (De Souza et al. 1997). However, malaria research mainly relies on mouse models to investigate the host immune response during malaria infection. Although these models cannot reflect all aspects of human infections, they allow the study of controlled experimental infections. Combating malaria due to the parasites resistance and immune dysregulation involves the use of integrated therapy and discovery of new anti-malarial drugs from different sources, especially from medicinal phytochemicals. The use of plant extracts and pure compounds derived from natural source has been the foundation for modern pharmaceutical compounds (Udodeme et al. 2016). Drugs in current use for malaria chemotherapy include artemisinin, from *Artemisia annua* (*Asteraceae*) of Chinese origin, and its semi-synthetic derivatives, artemether, artesunate and arteether. *S. jamaicensis* is found in scattered patches, with each patch hosting a small population of plants. This plant is a member of the family Verbenaceae commonly known as the blue porter weed (Idu et al. 2007). This plant mostly grows in the tropical regions of America, as well as in the subtropical forests of Africa, Asia, and Oceania. It is a seasonal shrub which produces flowers for a lengthy period during rainy seasons with floral characteristics such as bluish-violet flowers, no perceptible smell, narrow tubular corolla (Lakshmi and Raju 2011). This plant has been used traditionally as a cure for different ailments (Liew and Young 2016). It has been reported by several authors (Okokon et al. 2008; Bankole et al. 2016; Nardos and Makonnen 2017; Owolodun et al. 2019) that malaria parasites has developed resistance to antiplasmodial drugs and this has been a major challenge in control and treatment of the disease through chemotherapy, something that necessitates the continuous search for alternative antiplasmodial agents. The use of plants to treat malaria has been an age long activity though most of the plants used has not been scientifically evaluated. The present study aimed at evaluating the anti-malarial and immunomodulatory effects of Dichloromethane plant extracts of *Stachytarpheta jamaicensis* in mice infected with *Plasmodium berghei*.

Materials and methods

Collection and preparation of the plant materials

Stachytarpheta jamaicensis plant was collected from the Botanical garden of University of Ilorin, Ilorin, Kwara State, Nigeria. Identification and authentication was done at the Department of Plant Biology Herbarium, University of Ilorin. The leaves of *S. jamaicensis* were air-dried at room temperature. Dried leaves of *S. jamaicensis* were coarsely

powdered using mortar and pestle, followed by an electric mill. The powdered plant was weighed. Then the powdered plant materials were stored in a polythene bag and they were kept at room temperature for extraction.

Experimental animals

Forty Swiss albino mice, 6–8 weeks old with average weight 21.7 g were obtained from the Animal House of the Institute for Advanced Medical Research and Training (IMRAT), College of Medicine, University of Ibadan, Ibadan were used. They were housed in plastic cages and acclimatized for two weeks with perforated net-cover to allow for exchange of air with softwood shavings as beddings, kept in the Department of Zoology's animal house, University of Ilorin with free access to pellet diet and clean drinking water.

Parasites

Plasmodium berghei (ANKA strain) was obtained at IMRAT. Chloroquine sensitive *Plasmodium berghei* (ANKA strain) was used for induction of malaria in experimental mice. Mice previously infected with *P. berghei* were used as donors. The parasites were subsequently maintained in the laboratory by serial passage of blood from donor infected mice to naive one via intra-peritoneal (IP) route.

Drugs and reagents

Dihydro-artemisilin and piperazine (Pharmacology department, UIH), Dichloromethane, (DCM), distilled water, tween 80, DMSO, physiological saline, Heparin, buffered water, Giemsa stain and immersion oil were made use of. For phytochemical screening analysis, the following reagents were used; 5% FeCl₃, 10% FeCl₃, concentrated HCl, Conc. H₂SO₄, 50% H₂SO₄, 20% NaOH and Acetic anhydride. All reagents were analytically evaluated and obtained from certified suppliers.

Extraction of the plant materials

The solvent (dichloromethane) was distilled for purification. The powdered plants weighing 0.35 kg were soaked in distilled DCM in separate beakers for five days. The solvent DCM was used because it is denser than water, thus allows substances to properly dissolve in it. The extracts were decanted and filtered with litmus paper; the filtrates were concentrated to separate the extract from the solvent. The concentrated extracts were measured to determine the percentage yield. Finally, the dried extracts were then transferred into a glass container and refrigerated.

Inoculums' preparation

The parasitemia of the previously *P. berghei* infected donor mouse were determined. The mouse was anaesthetized with chloroform (diethylether), the blood from this mouse was collected via cardiac puncture into a test tube containing 0.5% Heparin which was added as an anticoagulant. The blood was then diluted with normal saline to give 1×10^2 infected red blood cells (IRBCs) in an injection volume of 0.2 ml for the suppressive test and 1×10^7 IRBCs in an injection volume of 0.2 ml for the curative test. Each mouse was then infected by injecting 0.2 ml of this diluted blood intra-peritoneally, which produced a steadily rising infection in mice.

Preparation of the extract and drugs

A stock solution of 1000 mg/kg was prepared by dissolving the extract in distilled water. The stock solution of the extract prepared was then stored in an air-tight container in the refrigerator at 40 °C. Subsequently, 200 mg/kg, 400 mg/kg and 600 mg/kg to be administered to the experimental animals were prepared from the stock solution by serial dilution. Piperaquine and dihydroartemisinin were also prepared according to the standard dosage of 18 mg/kg and 4 mg/kg respectively. Piperaquine was prepared with distilled water while dihydro-artemisinin with DMSO and distilled water in the ratio 1:9.

Grouping and dosing of animals

Forty albino mice were divided into two equal halves (A and B) to be used for the suppressive and curative studies respectively. Each half was divided into five groups which include I, II, III, IV and V and injected with blood containing infected RBC. Mice in groups I, II and III of the two halves were treated with 200 mg/kg, 400 mg/kg and 600 mg/kg doses of DCM leaf extracts of *S. jamaicensis* while mice in group IV were given distilled water and served as the negative control and group (V) mice were treated with the standard drugs (Piperaquine- Dihydro-artemisinin) and they functioned as positive control. Plant extracts and standard drugs were administered orally using a cannula to ensure safe administration.

Four-day suppressive antimalarial test

The standard *Plasmodium berghei* four-day suppressive test was done to determine the efficacy of the extracts by comparing blood parasitaemia which was observed by parasite counts and survival times of treated and untreated mice. The day of inoculation was defined as day zero (DO). The

infected animals were distributed randomly into five groups of four animals per cage and were treated once daily with the extract by oral route. The animals in group I received 200 mg/kg dose of the extract by oral route. Groups II and III received 400 mg/kg and 600 mg/kg, respectively. Group IV were given distilled water (negative control) while group V was treated with the standard drug (Piperaquine- Dihydro-artemisinin). Treatment commenced on Day 3 post-infection and the treatment groups received the administration of the plant extracts and the positive control group was administered the standard drugs for four consecutive days. Parasitemia were monitored on D3-D18 and subsequently on a 2-day interval until D35 and D30 for the suppressive and curative tests respectively. The anti-malarial activity of the extracts was determined using the Peter's 4-day suppressive test (Peter and Anatoli 1998). Survival rate of animals were monitored till day 14. On day 4 of the experiment, the animals' tail tip was cut to prepare the blood smears. The air-dried films were fixed with methanol and stained with Giemsa solution.

The percent parasitemia were determined by counting the number of infected erythrocytes per 1000 erythrocytes using microscope. In this study, in vivo antimalarial activity of the plant extract and the mouse survival were monitored over a period of three weeks.

The difference between the mean values of the control group (defined as 100%) and those of the experimental groups was calculated and expressed as percent suppression of parasite growth using the method of Devi et al. (2001) as shown in the equation below.

Suppression of parasite.

$$\% \text{ parasitemia} = \frac{\text{Number of infected RBCs}}{\text{Total number of RBCs}} \times 100$$

Collection of blood sample

Blood samples were collected by bleeding retro-orbitally from mice in each group on D7 post-infection, blood samples were also taken on D14 post-infection. The blood samples were collected in eppendorf tubes which were cold-centrifuged at 4 °C to allow separation of the serum from plasma. The blood plasma and serum were separated using micro-pipette into different eppendorf tubes and stored in -80 °C refrigerator for analysis of the various immune parameters with the use of an ELISA kit.

Phytochemical screening

Chemical tests for the screening and identification of bio-active chemical constituents of the medicinal plant were

carried out in aqueous extracts using the standard procedures as outlined by Trease and Evans (2002) and Sofowora (1993).

Quantitative analysis of phytochemical constituents.

Test for carbohydrates (reducing sugar)

Fehling's test:

5 ml of mixture of equal volumes of Fehling's solution A and B was added to 2 ml of test extract in a test tube. Mixture was boiled for a minute or two. Brick red precipitate of CuO (Copper (1) oxide) is indicative of a positive test.

Test for alkaloids

1 ml of 1% Hydrochloric acid (HCl) was added to 3 cm³ of test fraction in test tube. The solution was treated with a few drops of Meyer, Wagner and Drangendorff's reagents separately. Appearance of creamy white, reddish-brown and orange-brown in Mayer, Wagner and Drangendorff indicate the presence of alkaloids.

Test for tannin

Two (2) drops of 5% of FeCl₃ were added to the extract. A dirty green precipitate is indicative of a positive test.

Test for saponin

Emulsion test was carried out: 5 drops of olive was added to 3 cm³ to test extract in test tube and the mixture was shaken vigorously. A stable emulsion formed indicates the presence of saponin.

Test for flavonoids

2 ml of extract was treated with a few drops of 20% NaOH solution. Formation of intense yellow colouration which turns colourless on addition of dilute HCL acid indicates the presence flavonoid.

Test for phenolics

Two (2) drops of 10% FeCl₃ was added to 2 ml of test extract in test tube. Blue- green precipitate indicates the presence of phenolics.

Test for terpenoids (Salkowski test)

5 ml of extract mixed in 2 ml of chloroform and 3 ml of concentrated H₂SO₄ was carefully added to form a layer. A

reddish brown colour at the interface indicates the presence of terpenoids.

Test for steroid

2 ml of acetic anhydride was added to 0.5 g of extract with 2 ml of H₂SO₄. Colour changes from violet to blue or green is indicative of a positive test.

Test for Quinones

A small amount of extract was treated with concentrated H₂SO₄. Formation of a yellow precipitate indicates the presence of quinones.

ELISA test

Collection of blood for measurement of cytokine proteins in serum

To evaluate the immunomodulatory potential of the plant extracts, the analysis of pro-inflammatory and anti-inflammatory cytokines, blood was collected from the treatment and control groups of mice. Mice within each group were subjected to retro-orbital bleeding after pre-treatment and on day 3 and day 7 post-infection. The blood from the groups were allowed to clot for 30 min at 4 °C, and later cold-centrifuged at 12,000 rpm for 20 min. The sera was separated from the plasma and stored at −80 °C for subsequent ELISA (enzyme-linked immunosorbent assay) analysis for cytokine proteins.

Enzyme-linked immunosorbent assay

The levels of TGF-β and IFN-γ in chemotherapy and immunoprophylactic groups produced during pre-treatment and infection were measured with commercial Boster Immunoleader ELISA kits following the manufacturer's instructions.

Preparation of standard

For, both TGF-β and IFN-γ assays, standard solutions were prepared. To prepare 10,000 pg/ml of TGF- β and IFN-γ standard solutions, 1 ml of sample dilution buffer (from assay kit) was added to the standard reagent. To this solution, 0.9 ml of sample dilution buffer was added and the resulting solution represents TGF-β and IFN-γ standard solutions. From the respective standard solutions, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.2 pg/ml and 15.6 pg/ml were prepared via serial dilution.

Preparation of biotinylated TGF- β and IFN- γ antibody-working solution

The biotinylated antibodies for TGF- β and IFN- γ were diluted in 1:100 with the antibody dilution buffers; 0.1 ml was then aliquoted into wells of the microtitre plates.

Preparation of avidin-peroxidase complex (ABC)-working solution

The Avidin-Peroxidase Complex (ABC) was diluted in 1:100 with the ABC dilution buffer. From the diluents, 0.1 ml was aliquoted into wells of the microtitre plates.

TGF- β and IFN- γ assays

Ten microlitres of serum sample was transferred into each well of the TGF- β and IFN- γ pre-coated plates to which 90 μ l of sample diluents buffer was then added. To the remaining wells on the pre-coated plates, 0.1 ml of varying concentrations of TGF- β and IFN- γ standard solutions were added. A well with dilution buffer served as control. The plates were sealed with the cover and incubated at 37 °C for 90 min. After incubation, contents of the plates were discarded and the plates were blotted onto paper towels. To each well, 100 μ l of biotinylated (TGF- β and IFN- γ) antibody working solution was added and the plate incubated for 60 min at 37 °C. After incubation, the plates were spiked with 0.01 M Phosphate buffered saline (PBS) and then washed. The plates were then blotted with paper towels following washing. To each well, 0.1 ml of ABC working solution was added and incubated at 37 °C for 30 min. After incubation the plates were washed five times with the washing buffer. After washing, 90 μ l of tetramethylbenzidine (TMB) color developing agent was added to each well and the plates were incubated at 37 °C in the dark for 30 min. After incubation, the plates were monitored for a yellow color change (indicating antigen-antibody reaction), and 0.1 ml of TMB stop solution was added into each well to terminate the reaction. The optical density (OD) was read at 450 nm using Thermoscientific Multiskan Ex microplate reader. The relative OD values were calculated by subtracting the OD of zero well from the OD of each well. The standard curves were drawn and the concentrations of the cytokines (TGF- β and IFN- γ) were interpolated from the standard curves respectively.

Statistical analysis

Mean parasitaemia was determined in the control and treated groups. The mean parasitaemia was compared using the student's *t* test. Percent suppression of parasite growth of the

treated and control groups were compared using one-way ANOVA and two-tailed student's *t* test (Graph Pad Prism 4.0) with *P* < 0.05 being considered significant.

Results

Suppressive activity

Figure 1 illustrates the suppressive activity of Dichloromethane leaf extracts of *S. jamaicensis* against *P. berghei* ANKA in infected mice was examined in early infection (4-day suppressive test). The extract at 200 mg/kg body weight of mice gave 52.9% chemosuppression when compared with the untreated (negative) control group on Day 11 post-infection. At the dose of 600 mg/kg, it induced the highest chemosuppression of 67.6%. Positive control group (administered with piperazine and dihydroartemisinin) had a chemosuppression of 100% (Table 1). Percentage chemosuppressive effect was observed to be dependent on increasing concentrations of the extract. The results showed that the extracts reduced parasitaemia in a dose-dependent manner, ranging from 1.1 to 1.7%. The mean parasitaemia in positive control group was 0%, while the negative control group had the highest level of parasitemia of 3.87%.

Qualitative phytochemical screening of *S. jamaicensis*

The result of the qualitative phytochemical screening of the leaves of *S. jamaicensis* revealed the presence of alkaloid only (Table 2).

Mean survival proportion

Figure 2 shows the mean survival period for both untreated and treated (with extracts and standard drug) *P. berghei* infected mice in a day 4-suppressive test. All the groups (both treated and untreated) experienced 100% survival till Day 5 post-infection. On Day 6, the survival rate for all treatment groups was still at 100%, except 400 mg/kg extract group that showed a decline in survival to 83%. On Day 8, 94.4%, 83%, 80% and 94.4% survival rates were observed for 200 mg/kg, 400 mg/kg, 600 mg/kg and negative control groups respectively, with the positive control group observed to have maintained 100% survival rate on the same day. On Day 10, positive control group and groups treated with 200 mg/kg, 400 mg/kg and 600 mg/kg extracts experienced decline in survivorship, having survival rates of 89%, 55.6%, 80% and 93.75%, respectively, while the negative control was still at constant proportion of 94.4%. On Day 12, both negative and positive control groups

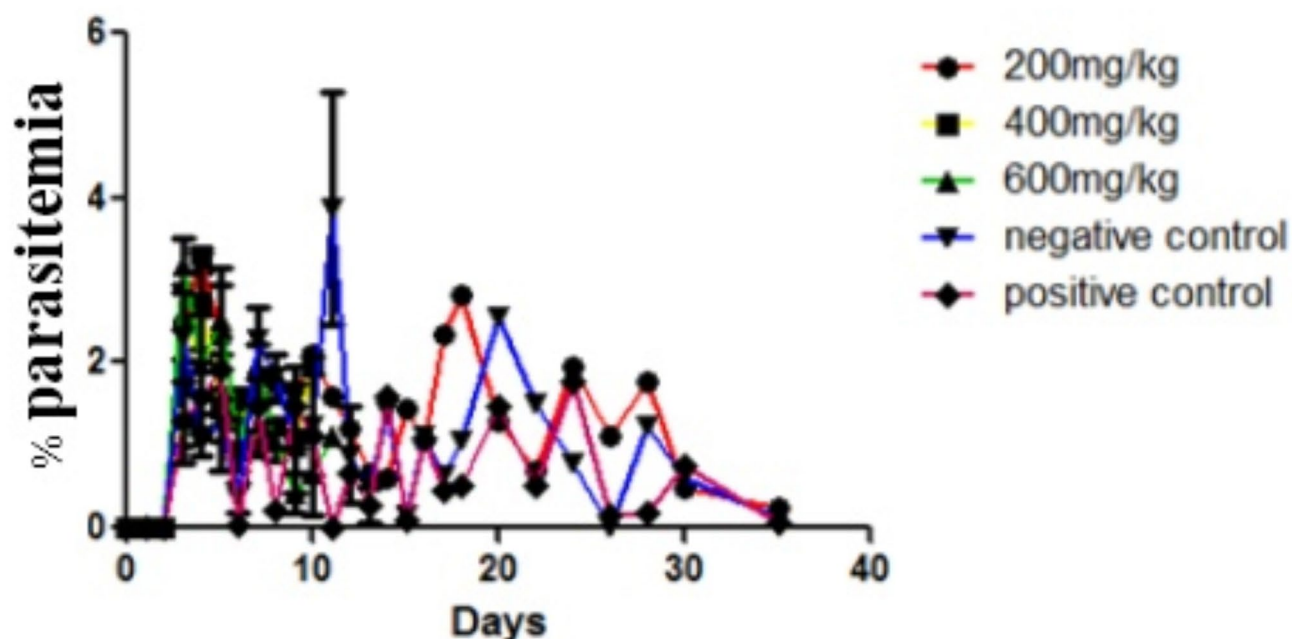


Fig. 1 Percentage parasitemia of mice infected with *P. berghei* in a suppressive test

Table 1 Antimalarial activity of dichloromethane extracts of *Stachytarpheta jamaicensis* in mice infected with *Plasmodium berghei* in early infection (4- day suppressive test)

Extract/drug	Dose (mg/kg/day)	Average parasitaemia	%chemo-suppression
<i>Stachytarpheta jamaicensis</i>	200	1.7 ± 0.17	52.9
	600	1.1 ± 0.03	67.6
Dihydroartemisinin and piperazine	0.2 ml	0	100
Control(water and tween 80)	0.2 ml	3.87 ± 1.41	0

Table 2 Qualitative phytochemical screening results leaves of *S. jamaicensis*

Phytochemicals	Activity
Alkaloids	Present
Tanins	Absent
Saponin	Absent
Flavonoids	Absent
Phenolics	Absent
Steroids	Absent
Reducing sugar	Absent
Terpenoids	Absent

experienced decline in survival rates to 88% and 87.5% respectively, while the extract group showed no decline in survival. The constant survival rate of 89% was observed for the 200 mg/kg till the end of the experiment.

Curative test

Figure 3 shows the percent parasitemia of *P. berghei* infected mice. Manifestation of malaria infection was observed in *P. berghei* infected mice on Day 3 as depicted by percent parasitemia. While untreated mice (negative group) showed increasing level of parasitaemia, drug and extract treated groups showed curative activity which was manifested on Day 6. This was substantiated by the decrease in parasitemia level of the groups treated with standard drugs (0.68%), 200 mg/kg (1.2%) and 600 mg/kg (1.27%) when compared with the untreated group with parasitemia level of 6.4%. However, slight increase in parasitemia was observed in 600 mg/kg extract treated group from 5% on Day 8 to 6% on Day 10. On the other hand, 200 mg/kg extract group showed a steady parasitaemia level till Day 8 with elevated parasitaemia (5%) on Day 10. From Day 11, there was continuous decrease in parasitemia level of mice treated with 200 mg/kg extract up to 0.5% level at the point of termination of the experiment on Day 30. This compared favorably

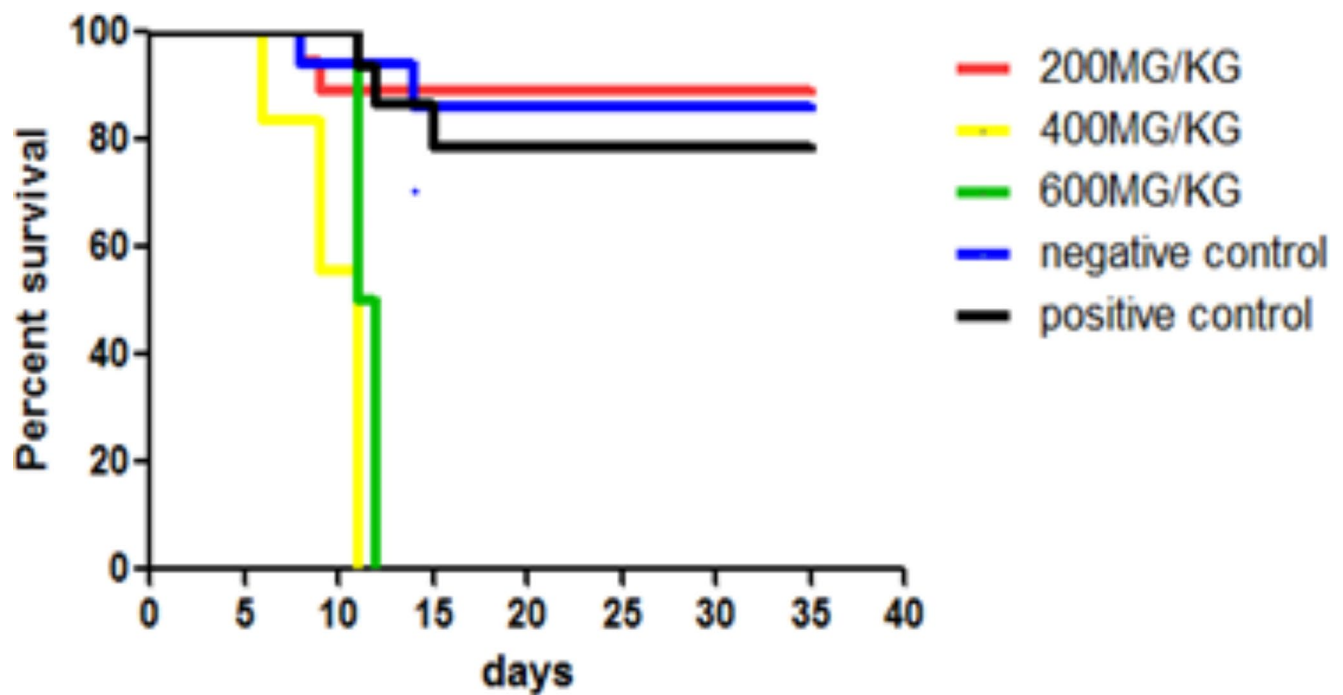


Fig. 2 Percentage survival rate of treated and untreated mice

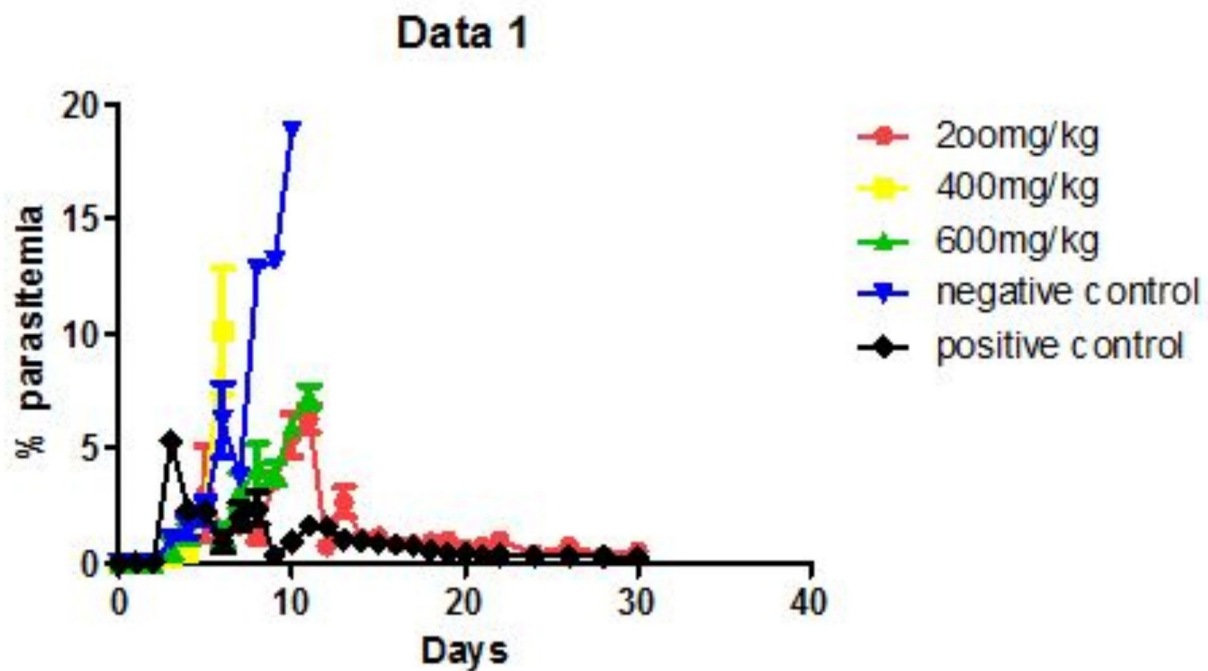


Fig. 3 Percentage parasitemia in *P. berghei* infected mice in a curative test

Fig. 4 Percentage survival of treated and untreated mice

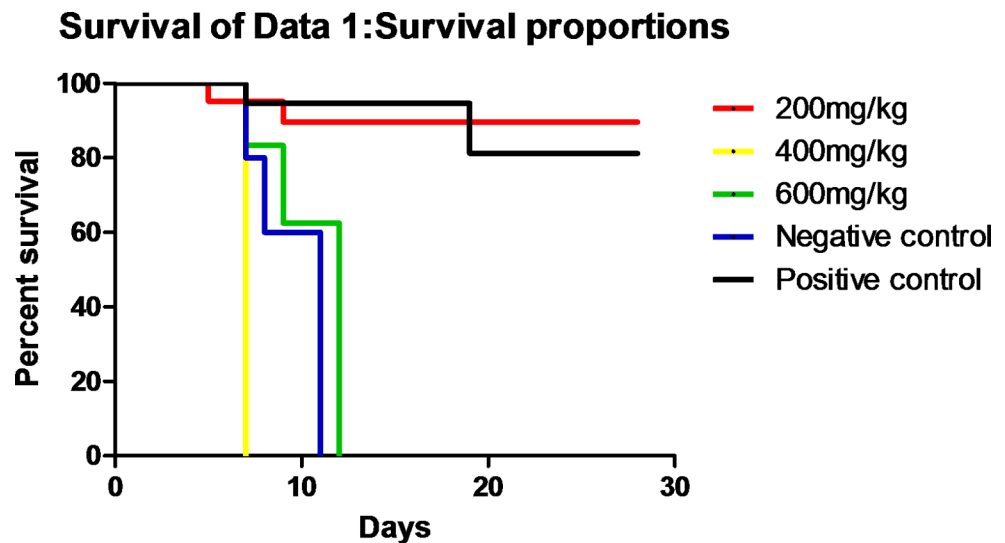
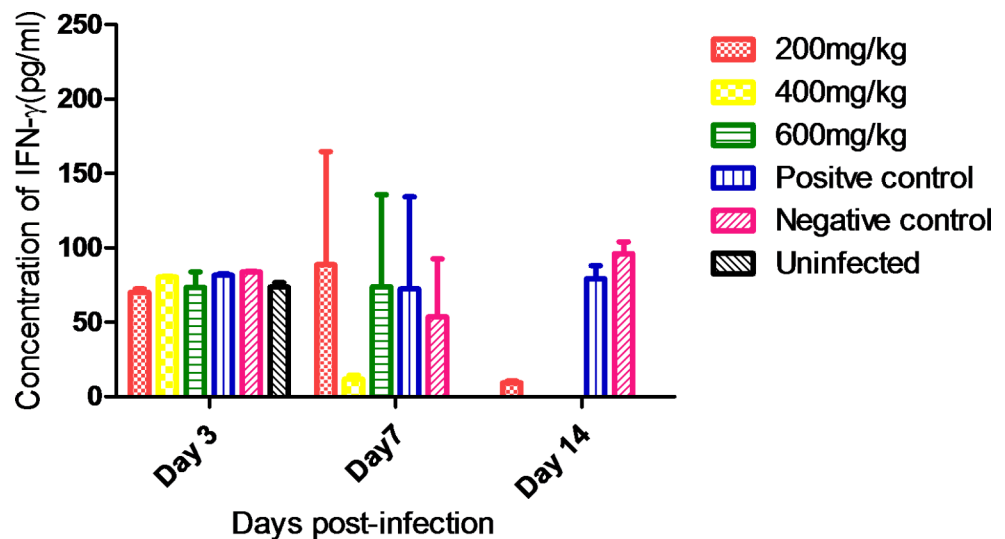


Fig. 5 Effects of *S. jamaicensis* on serum levels of IFN- γ in *P. berghei*-infected mice



with the positive control group which showed total parasite clearance.

Mean survival proportion

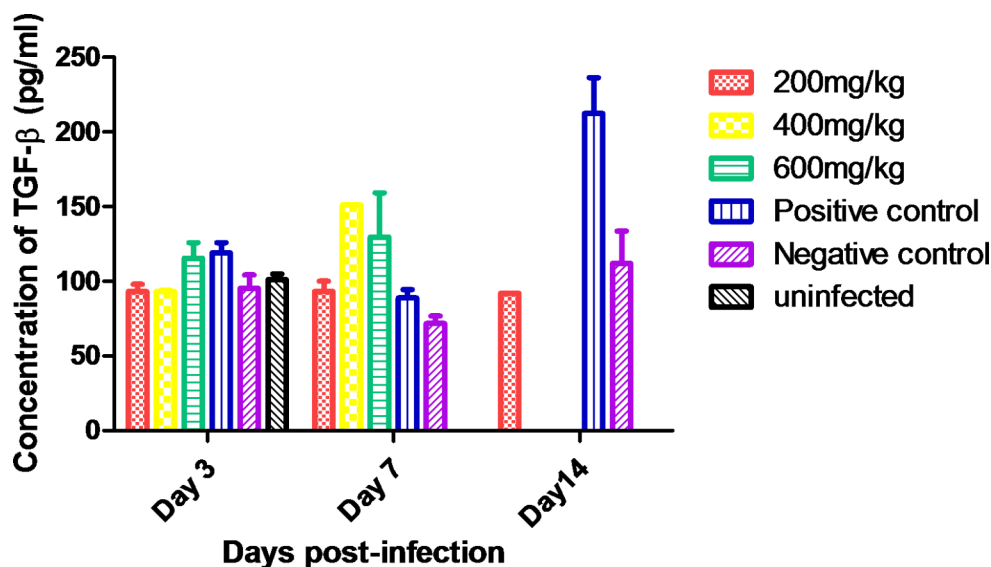
Figure 4 shows the mean survival rate of treated and untreated *P. berghei* infected mice. Untreated group experienced continuous decline in survival and total mortality on Day 11. All experimental groups experienced 100% survival till Day 5, except 200 mg/kg extract group which showed a decline in survival to a proportion of 95% on Day 5. The survival rate for 200 mg/kg group remained the same on Day 7, while drug and 600 mg/kg extract treated groups experienced decline in survival rates (95% and 83% respectively), while 400 mg/kg extract group experienced total mortality without a prior decline in survival. Meanwhile, 200 mg/kg extract-treated group and positive control experienced prolonged survival of 90% and 95% rates

respectively from Day 12, this was observed throughout the study.

Concentration of IFN- γ (pro-inflammatory cytokine) in *P. berghei* - infected mice in a suppressive study using *S. jamaicensis*

Figure 5 shows the concentration of IFN- γ in serum of *P. berghei* infected mice collected from Day 3 to Day 14 at intervals. There was early production of IFN- γ in all groups, however on Day 3, IFN- γ levels in all groups and uninfected mice were not significantly different, with concentrations of 70.15 pg/ml, 80.35 pg/ml, 73.45 pg/ml, 81.5 pg/ml and 83.9 pg/ml observed in 200 mg/kg, 400 mg/kg, 600 mg/kg, positive and negative control -+groups respectively and 73.55 pg/ml in uninfected mice. As infection progressed on Day 7, while 200 mg/kg experienced increase in IFN- γ level to 88.6 pg/ml. The IFN- γ level of 73 pg/ml was maintained

Fig. 6 Effects of *S. jamaicensis* on serum levels for TGF- β in *P. berghei*-infected mice



in 600 mg/kg while the 400 mg/kg, positive and negative control groups experienced decline in IFN- γ levels occurred to 11.75 pg/ml, 72.3 pg/ml and 53.6 pg/ml respectively. On day 14, while 400 mg/kg group had experienced total mortality prior to this day, there was significant reduction in the IFN- γ concentration in 200 mg/kg to 9.25 pg/ml. While both positive and negative control group experienced increase in IFN- γ levels at 79.3 pg/ml and 96.1 pg/ml respectively.

Concentration of TGF- β (anti-inflammatory cytokine) in *P. berghei*-infected mice in a suppressive study using *S. jamaicensis*

Figure 6 shows the concentration of TGF- β in serum of *P. berghei* infected mice collected from Day3 to Day14 at intervals, there was early production of TGF- β in all groups. However prior to treatment on Day 3, 200 mg/kg, 400 mg/kg, negative and uninfected groups did not show significant difference in levels of TGF- β at 93.2 pg/ml, 95.4 pg/ml and 101.1 pg/ml concentrations respectively, while its levels in positive and 600 mg/kg were different at 119.15 pg/ml and 115.6 pg/ml respectively. As at Day 7 post-infection, while the TGF- β level in 200 mg/kg group was still constant, its levels in negative and positive control groups decreased to 71.8 pg/ml and 88.8 pg/ml respectively but 400 mg/kg and 600 mg/kg extract- treated groups experienced increase in TGF- β levels of 151.2 pg/ml and 129.6 pg/ml respectively. On Day 14, 400 mg/kg and 600 mg/kg groups had experienced 100% mortality prior to the day, while the TGF- β level in serum of 200 mg/kg group was not significantly different from the level on day 7. Both the negative control and positive control groups experienced increase in serum level of TGF- β and the highest level of 212.45 pg/ml was recorded in positive control group.

Discussion

The emergence of widespread resistance of Plasmodium species to most antimalarial drugs has led to a more vigorous and concerted research in traditional medicinal plants for the treatment of malaria. It is important to constantly screen medicinal plants in order to determine their potential as new antimalarial compound. *S. jamaicensis* has been an important medicinal plant with great medicinal properties in traditional and folk medicinal systems but has not been scientifically proven in the use against malaria parasite (Liew and Young 2016). The outcome of this study indicates that di chloromethane leaf extract of *S. jamaicensis* has intrinsic antimalarial activity considering its chemosuppressive ability and prolong survival rate in the infected mice treated with 200 mg/kg and 600 mg/kg b.w of the extract. Although, negative control group was also found to have survived till the end of the study, this could be attributed to low inoculum size which results to development of immunity, as it has been reported by Marsh and Kinyanjui (2006) that with many years of exposure, individuals living in endemic areas develop clinical immunity to disease, which is characterised by low parasitemia levels. The chemosuppressive activity of *S. jamaicensis* compared favourably with the standard drugs used. The chemosuppression produced by the extract on Day11 was remarkable and dose-dependent with the highest dose (600 mg/kg) having the best activity. The findings of this work is in agreement with the report of Okokon et al. (2008) who observed that at 90, 180, and 270 mg/kg bw leaf extract of *Stachytarpheta cayennensis* produced a significant ($P < 0.05$) antiplasmodial activity as evident from the chemosuppression in a 4-day early infection test. The same leaf extract also exhibited significant curative effect in established infection which was comparable to the standard

drug which was demonstrated in the mean survival time. The dichromethane extract of *S. jamaicensis* exhibited significant schizontocidal activity comparable to that of the standard drug, piperazine-dihydro-artemisinin. The antiparasitic activity confirms its folkloric use in the treatment of malaria. The result of the qualitative phytochemical screening test in this study showed the presence of alkaloids only in *S. jamaicensis*. This is in agreement with the study of Vikasari et al. (2015) who reported the presence of alkaloids in the aqueous leaf extract of *S. jamaicensis* and other non-alkaloid secondary metabolites such as steroids, flavonoids, triterpene, monoterpene and saponins. The absence of non alkaloids reported in this work may be attributed to differences in methods of extraction and location of the plant. The mechanism of action of plant extracts depends on their phytochemical constituents (Ayoola et al. 2008). The antimalarial activity of *S. jamaicensis* reported in this work may therefore be attributed to the presence of alkaloids. Alkaloids, as reported by Nergiz and Otles (1993), could confer protection against malaria infection by inhibiting protein-synthesis in *Plasmodium falciparum*. Reporting only alkaloid in the phytochemical screening is novel as no other author has reported same. Most alkaloids exert some definite pharmacological actions. Despite all the medicinal importance of this plant, throughout this study, there are no prior reports on its use as an antimalarial drug. However, Ezenwa and Idu (2015) did not report the presence of alkaloid in this plant extract. The specie and method of extraction might have accounted for the differences observed by different authors.

Early proinflammatory cytokine responses are required to mediate protective immunity. Suppressive study of *S. jamaicensis* in *P. berghei* infected mice showed early production of IFN- γ prior to treatment and the levels in all groups and uninfected mice were high and not significantly different. This may be responsible for the low parasitemia observed in all groups as it has been reported by D'Ombrain et al. (2008) that an early production of pro-inflammatory cytokines such as tumor necrosis factor TNF- α and IFN- γ promotes parasite clearance. The decline IFN- γ level found in 400 mg/kg group as infection progressed on Day 7 may be attributed to the increase TGF- β observed in the group and this may be responsible for their total mortality as Omer and Riley (1998) reported that high levels of TGF- β in early infection prevents Th-1-cell mediated immunity through inhibition of pro-inflammatory cytokines such as IFN- γ and TNF- α which suppress a rapid growth of parasite, thus resulting in high parasite burden. The slightly elevated IFN- γ level observed in the 200 mg/kg group during early infection could be responsible for the low parasitemia as D'Ombrain et al. (2008) reported that an early production of pro-inflammatory cytokines such as TNF- α

and IFN- γ inhibits parasite growth. Although high IFN- γ level was observed in the negative control during late infection, low parasitemia and prolonged survival observed in this group could be attributed to the low parasite density inoculated in the mice, as well as the higher TGF- β level (compared to IFN), resulting in reduced IFN- γ to TGF- β ratio. Diana and Louis (2010) reported that enhanced levels of T cell activation and IFN- γ production in spleens of *P. berghei*-ANKA-infected mice correlated with increased resistance to infection.

Anti-inflammatory cytokines are required to down-regulate the pathological effect that result from over-production of pro-inflammatory cytokines (Artavanis et al. 2003). The balance between pro- and anti-inflammatory cytokines plays a crucial role in modulating the host immune responses and pathogenesis in malaria infection. The results of this study showed that concentration of TGF- β was found to be high during early infection prior to treatment in all groups. This could have been responsible for the low parasitemia level found in the mice, as early production of TGF- β has been reported by Ferrante et al. (1990) to confer protection by activating macrophages and monocytes which phagocytose infected red blood cells, thus killing the parasites. As infection progressed, the concentration of TGF- β in extract-treated groups was found to have increased but not dose-dependent with 400 mg/kg extract group observed to have the highest concentration. This could have resulted into their total mortality as it has been reported by Omer and Riley (1998) that high level of TGF- β in early infection prevents Th-1-cell mediated immunity through inhibition of pro-inflammatory cytokines such as IFN- γ and TNF- α from suppressing a rapid growth of parasite. Although, negative control group was also found to have survived till the end of the study, this could be attributed to the low parasite density inoculation into the mice, which may have resulted in development of immunity, as it has been reported by Marsh and Kinyanjui (2006) that with many years of exposure, individuals living in endemic areas develop clinical immunity to the disease, which is characterised by low parasitemia levels. The constant TGF- β concentration maintained by 200 mg/kg extract group at the late phase of the study which was not significantly different from the concentration at the beginning could have translated into their prolonged survival. Sustained and strong TGF- β response leads to abrogation of mortality and resolution of infection (Omer and Riley 1998). Also, increased TGF- β levels observed in the positive control group at the late phase of the study could be responsible for their prolonged survival and low parasitemia, this is in agreement with the report of Esamai et al. (2003), which stated that high serum concentrations of TGF- β in murine model and human inversely correlated with severe malaria.

The result of this study is in accordance with the report of Vikasari et al. (2015) which stated that the water extract *S. jamaicensis* showed an immunosuppressive activity with the use of carbon clearance assay. However, the immune modulatory effect was attributed to the presence of flavonoid which had the ability to reduce the level of TNF- α , a pro-inflammatory cytokine, although phytochemical screening of this study did not indicate the presence of flavonoid.

Conclusion

With increasing resistance developed by malaria parasite to antimalarial drugs, there is a dire need for new and effective drugs. This study established that *S. jamaicensis* have promising anti-plasmodial properties. This may be attributed to the presence of alkaloids, a medicinal phytochemical reported to confer protection against malaria infection by inhibiting protein synthesis in *Plasmodium* spp. *S. jamaicensis* at 200 mg/kg dose mediated parasite clearance and improved survival throughout the study. The intense and sustained TGF- β level stimulated by *S. jamaicensis* at 200 mg/kg dose may have translated into the prolonged survival observed in the mice. *S. jamaicensis* modulated both pro and anti-inflammatory responses in early and late infections, thus preventing pathological outcomes associated with excessive expression of the pro-inflammatory (IFN- γ) and anti-inflammatory (TGF- β) cytokines. *S. jamaicensis* may therefore be explored as alternatives for in clinical trials for the development of novel antimalarial and immunomodulatory drugs.

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Authors contribution OAO conceptualized and designed the study. OAO and KOL carried out the laboratory work. ECA wrote the first draft. OAO and KOL analyzed the data. All the authors read through and made inputs.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval Guidelines on the use of animals was fully followed throughout the study. Ethical approval was sort and approval gotten from the University Ethical Committee.

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