Seeds of *Asparagus schweinfurthii* (Liliaceae) Protects Mice from Fatal Murine Experimental Cerebral Malaria

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Abstract: Most people die in Africa due to plasmodium with lead to complication cases principally in *Plasmodium falciparum* infection. For unknown reasons, *P. falciparum* progress to severe malaria resulting in high mortality rate in children mostly in sub-Saharan Africa. Difficulties in severe malaria therapy result in complexity to predict early symptoms and when people can develop severe malaria. For adjunctive therapy, New drug are need, to improve outcome in cerebral malaria. *Asparagus schweinfurthii* is a perennial plants with multiple proprieties including antiprotozoal, antioxidant and anti-inflammatory properties with emerging antimalarial potential. The influence of *A. schweinfurthii* administration to rescue and prior to *Plasmodium berghei* infection on parasitemia induction is there reported. Extract of *A. schweinfurthii* was administered at doses of 38.12, 19.06, 9.5 and 3.8 mg/kg to mice (15–18 g) 72h before intraperitoneal infection with *P. berghei* (prophylaxis test) or days 6 post-infection (pi) (rescues therapy). Parasitemia induction and progression, clinical signs of experimental cerebral malaria and temperature were recorded during sub-chronic studies (18 days). Administration of *A. schweinfurthii* in pre-infected animals preserved significantly temperature loss (p<0.001). The plant extract also abolish significantly (p<0.001) parasitemia and clinical signs of experimental cerebral malaria while oral Cofantrine (10 mg/kg) failed to prevent malaria induction. Administered orally, prior to the infestation of mice with *plasmodium berghei*; *A. schweinfurthii* administration influenced parasitemia patency and parasitemia progression as well as temperature loss. This may suggest possible chemoprophylaxis effects of *A. schweinfurthii* on malaria parasite.

Keywords: *Asparagus schweinfurthii*, Cerebral Malaria, *Plasmodium berghei*, Prophylaxis Treatment

1. Introduction

Severe Complications of malaria are principally characterized by neurological symptoms such as convulsion and coma. These symptoms are directly linked with neurodegenerative or brain inflammation [1] which causes neurological and cognitive impairment in survived children [2]. The first line in therapy study is, to determine precisely, the moment where appropriate treatment can prevent development of cerebral malaria. *Plasmodium berghei* and mice are currently used as models for cerebral malaria experimental study and therapy reagents before Human utilization [3].

In Africa, many plants have been used in chemoprophylaxis or in suppressive of parasites from blood vessels [4]. Drugs used as prophylaxis therapy need to be longer half-lives [5, 6]. The plant derived drugs need also to be safe and tolerated by the consumers [7, 8]. For example, *Artemesia annua* infusion preserved cerebral malaria complication associated with *P. falciparum* [9]. However it is necessary to detect news drugs with higher half-life than *A. annua* infusion [10].

So plants in African flora, with antimalarial properties are candidate for antimalarial drugs research [11, 12]. Many studies have been conducted on traditionally claimed medicinal plants in Cameroon [13]. This is because they have been part of human life since immemorial time; and a number of plant products have been in extensive use in ethno-medicine [14]. *Asparagus* are used in folk medicine for their antibacterial, antiprotozoal and anti-inflammatory proprieties. Among innumerable species, *A. schweinfurthii* is used in traditional medicine known under the names of *A.
abyssinicus, A. flagellaris. This plant was described by Kunth in 1850 as a Senegambia collection. Cladodes are the main ingredient of the plant which combat Guinea worm and in many countries to treat eyesight troubles, stitch, syphilis, gonorrhea [15]. In Senegal and in Eastern Africa, macerated root is gaggled against throat troubles [16]. In Ethiopia pounded branches mixed with butter are used as an ointment for hemorrhoids treatment [17]. An embrocation is used in Senegal against rheumatism. In Zimbabwe his infusion is used to manage diabetes. In Côte d’Ivoire, Burkina Faso and Nigeria a root macerate is used against earache and hematuria [18] and to cure coughs in Kenya [19], in Eastern Africa the branches, stems or roots are pounded, soaked in water and the infusion drunk 2–3 times a day to restore mental disturbance [20] and antiprotozoal in Cameroun [21].

2. Material and Methods

2.1. Plant Material and Extraction

Seeds of A. schweinfurthii were collect around Ngaoundere III sub-division in the Vina division Adamawa-Region of Cameroon in August. At this moment, corresponded to the period of fruits maturations and seeds were removed to the bay. After 20 days of drying, the powder obtained after crushing seeds on a stone was used for the preparation of the decoction.

2.2. Acute Toxicity

Swiss albino mice, fasted overnight, were used for the acute toxicity study. Following the period of fasting, the animals were weighed and 5, 50, 300 and 2000 mg/kg of the aqueous extract were administered by oral gavage. Food was then withheld for further 1 to 2 h. Firstly; one mouse was treated and observed continuously for the first 30 min and intermittently for 4 h during a period of 24 h. Since no death was observed, the same dose was given to another three female mice. Global behavioral changes like loss of appetite, hair erection, lacrimation, tremors, convulsions, mortality and other signs of toxic manifestation were observed for 14 days after administration of the extract [22].

2.3. Murine Malaria Model

Parasite Inoculation

Albino mice previously infected with P. berghei and having different levels of parasitemia (30-37%) were used as donors. Donor mice were placed in closed chamber and euthanized with inhalation of anesthetia gas and infected blood was collected by jugular vein into heparinized vacutainer tube containing 0.5% trisodium citrate. The blood was then diluted in normal saline (0.9%) based on parasitemia level of the donor mice and the red blood cell (RBC) count of normal mice so that the final suspension would contain about 1×10⁷ parasitized red blood cells (PRBCs) in every 0.2 mL suspension. Each mouse used in the study was infected intraperitoneally with 0.2 mL infected blood containing about 1×10⁷ P. berghei parasitized RBCs.

2.4. Clinical Assessment of ECM

All animals were checked several times daily for Parasitemia and Experimental Cerebral malaria (ECM) symptoms. For better estimation of the overall clinical status of mice during infection, simple behavioral tests (transfer arousal, locomotor activity, tail elevation, wire maneuver, contact righting reflex, and righting in arena) adapted from the SmithKline Beecham, Harwell, Imperial College, Royal London Hospital, phenotype assessment (SHIRPA) protocol [23] were used. Infected mice display signs of ECM at day 5 or 6 post infection [23]. ECM is defined as the presentation of one or more signs of neurological deficit including ataxia, convulsions, limb paralysis, poor righting reflex, roll-over and coma [24]. Presentation of these signs were evaluated and scored to better assess severity of ECM in mice [23]. The development of ECM was assessed using the SHIRPA protocol. Mice exhibiting early signs of ECM at score ≥10 on the SHIRPA, always. Oral administration of Asp 38.12, 19.06, 9.5, 3.8 mg/kg and Cofantrine Adults (10 mg/kg) in distilled water was given to mice in different experimental group. Any signs of illness were quantified using arbitrary scale and recorded as either absent (0), mild (1), moderate (2) or severe (3). Infection in the mice was allowed to continue until day 12 infection starting at days 3 pi.

2.5. Effect of Asparagus Schweinfurthii on Established Plasmodium Berghei Infection

Four doses of A. schweinfurthii seeds aqueous extract was prepared daily 38.12, 19.06, 9.5 and 3.8 mg/Kg during the examinations. Thirty mice of 1×10⁶ of P. berghei were inoculated on the first day to evaluate the curative antimalarial properties of A. schweinfurthii. The mice were assigned to five groups (n = 5). At day 6 post infection, the animals were treated once daily with four doses of A. schweinfurthii (38.12, 19.06, 9.5 and 3.8 mg/kg p.o.) (groups1–4), Infected mice treat distilled water (negative Control: group 5) and 10 mL/kg p.o. Distilled water (Non-infected control NIC: group 6) for 3 days. To determine the daily parasitemia level, about three drops of blood were collected from the tail of each mouse and smeared on to a microscope slide to make a thin film. The thin film was prepared from two drops of blood obtained from the tail of the mice. The smears were with 10% Geimsa, and examined under microscope (×100 magnification). The parasitemia has been determined by counting. On the day 18, two animals from each treatment group were sacrificed and the brains were taken for histopathological assay. The tissue was embedded in paraffin; 8µm sections were cut on a microtome (Bright 5040) and processed for routine haematoxylin-eosin staining. Slides of tissue sections were observed using trinocular clinical light microscope with a digital camera (Olympus CX1, Japan) connected to a computer. Micrographs of the tissue were generated using the ×10 objective lens for further analysis. The following scores were used to grade the degree of histopathological changes or lesions observed in the organs: not present (−), very mild (+),
mild (++), moderate (+++), and severe (++++).

2.6. Prophylactic Activity of Asparagus Schweinfurthii on P. berghei Infection

A. Schweinfurthii was further assayed for its prophylactic activity against P. berghei infection using the method described by Peters. The mice were randomly assigned to seven groups (n = 5) and pretreated orally with 38.12, 19.06, 9.5 and 3.8 mg/kg/day Cofantrine: Artheneter/Lumefantrine (the reference drug), and 10 mL/kg/day of distilled water for infected control (IC) and non-infected control (NIC). Treatment was continued for 3 consecutive days. At the day fourth, all mice has been infected with $1 \times 10^6$ P. berghei. Seventy-two hours later, blood smears has been prepared from the tail. Density of parasite count for all the treatment groups were determined.

2.7. Effect of Asparagus Schweinfurthii on Temperature During Experimental Cerebral Malaria

For better estimation of severity of malaria induction and efficacy of treatment, Body temperature was measured with a digital thermometer (Quick-check; Polylabo, Strasbourg, France). The thermometer is inserted into the rectum and read when the readout stabilized (after 20s) before and after treatment.

2.8. Statistical Analysis

Graph Pad Prism for Windows version 4.03 (Graph Pad Software, San Diego, CA, USA) was used for all statistical analyses, and was considered statistically significant at level of 5%. All data were expressed as mean±SEM. The time-course curves were subjected to two-way (treatment×time) repeated measures analysis of variance (ANOVA) with Turkey’s post hoc test.

3. Results

3.1. Acute Oral Toxicity Test

The acute oral toxicity test of A. Schweinfurthii indicated that neither aqueous extract of the plant caused gross behavioral changes and mortality within 24 h as well as in the following 14 days, indicating that the LD50 values of the extract were greater than 2000 mg/kg in mice. A. Schweinfurthii seeds extract, as prepared in this present study, can therefore be classified as category 5 and considered non-toxic orally, according to the OECD’s Globally Harmonized System of Classification [22]. Based on these results, antimalarial activity in ECM was assessed at different doses tested in this work.

3.2. Asparagus Schweinfurthii Reduces Clinical Symptoms of ECM

The score in the Asp-treated mice was significantly (P< 0.0001) less than that in the Control infected non treated mice (CINT) in later stages of infection (Figure 1). Results also showed significant (P< 0.0001) differences between the Asp 38.12, 19.06, 9.5 and 3.8 treated mice and the CINT groups at 8 to 12 days p.i. The value was less than 5 points in the Asparagus-treated groups on day 12 p.i. (Figure 1). In contrast to PbA-infected mice treat with distilled water (CINT) that developed ECM on day 8, all groups treated with aqueous extract were completely protected against ECM upon infection with PbA (Figure 1), without showing any symptoms of disease.

![Figure 1](image-url). Average of clinical score of SHIRPA during rescues therapy administered in day 6 post-infection (pi). Asp 38.12 to 3.8: doses 38.12, 19.06, 9.5 and 3.8 of Asparagus schweinfurthii. CS: Uninfected control, CINT: Control infected and untreated. *p < 0.05; ** p < 0.01; ***p < 0.001, difference between Asp, Cs treated group to Control infected and untreated (CINT). using ANOVA: Turkey test. Score ≥ 10: Mice with clinical signs of Experimental cerebral malaria (ECM).
3.3. Pretreatment Mice with Asparagus Schweinfurthii Promotes Resistance to Experimental Cerebral Malaria ECM

To determine if the mice in tested group were succumbing to *P. berghei* infection in our challenges as a result of ECM, mice were injected with *P. berghei* and symptoms of ECM were scored according to severity, from 0 (no symptoms) to 3 (death). Severe clinical symptoms were observed in group namely CINT, with most reduce locomotors activity, whereas group infected and treated with aqueous extract of *A. schweinfurthii* remained asymptomatic for the length of the experiment. At day 10 pi, mice treated by Asp 38.12 presented similar score to CS pi (Figure 2).

![Figure 2](image.png)

*Figure 2. Average of clinical score of SHIRPA during prophylaxis treatment 3 days prior infection mice by Plasmodium berghei. Asp 38. 12 to 3. 8: doses 38.12, 19.06, 9.5, 3.8 mg/Kg of Asparagus schweinfurthii. CS: Uninfected control. CINT: Control infected no-treated. *p < 0.05; ** p < 0.01, ***p < 0.001, difference between Asp, Cs treated group to Control infected and untreated (CINT) and COF: Cofantrine. using ANOVA: Turkey test. Mice with clinical signs of Experimental cerebral malaria (ECM).*

3.4. Asparagus Schweinfurthii Protected Mice from ECM Development Upon Blood-stage Infection with PbA

We tested the capacity of *A. schweinfurthii* to act therapeutically and cure an established PbA infection. For this, treatment of PbA-infected mice with *A. schweinfurthii* was performed daily starting at day 6 post-infection (pi) and continuing to day 8 with disease outcome monitored even when treatment was initiated later in the course of infection up to day 6 pi (Figure 3a). The protective effect of *A. schweinfurthii* was correlated with length of treatment and total dose received (Figure 3a) and was associated with a significant (p < 0.0001) reduction in blood parasitemia (measured at 8, 10 and 12 day pi; Figure 3a). Interestingly, in Asparagus–treated animals, parasitemia measured at day 8 was lower than at day 6, indicating that continued treatment with *A. schweinfurthii* caused a reduction of parasitemia from the peak measured at day 6 (Figure 3a). Finally, the therapeutic effect of *A. schweinfurthii* was found to be dose-dependent at day 10: animals dosed daily (starting at day 6 and continuing to day 8) with *A. schweinfurthii* at either 38.12, 19.06, 9.5, 3.8 mg/kg were protected against lethal PbA-induced CM, while animals receiving dose of cofantrine 10 mg/kg were susceptible as untreated controls (Figure 3a). Parasitemia levels were also affected in a non-dose-dependent manner (Figure 3a) at day12. These data suggest that therapeutic intervention with *A. schweinfurthii* can prevent and reduce significantly ECM model. The results of the study showed that *A. schweinfurthii* aqueous extract displayed a significant decrease in parasite count compared to that of the vehicle treated mice. In addition, it is important to note that the extract non-dose-dependently suppressed the malaria parasite significantly at all tested doses (3.8 and 9.5, 19.06 and 38.12 mg/kg (p < 0.0001).

3.5. Effect on Rectal Temperature in Rescue or Suppressive Test

The extract at the higher dose (38. 12 mg/kg) preserved a significant decrease (P=0.0015) and middle dose (Asp 19. 06, Asp 9. 5 mg/kg) (P< 0.05) in rectal temperature of *P. berghei*-infected mice (Figure 3b). Likewise, the lower dose (Asp 3. 8 mg/kg) showed a considerable (P<0.0001) attenuation in reduction of rectal temperature in the study’s mice. In experiment, the mice that developed ECM showed neurological signs and early hypothermia (Figure 3b).
3.6. Prophylactic Activities of A. schweinfurthii on P. berghei Infection in Mice

The potential antimalarial activity of *A. schweinfurthii* was assessed in vivo in a murine model of ECM induced by infection with PbA. In this model, mice infected with PbA develop blood-stage parasitemia detectable within 3 days. Using a prophylactic treatment regimen where mice received daily dosing for 3 days before infestation, *A. schweinfurthii* extract demonstrated at day 6 pi a substantial (P=0.0002) prophylactic action at the highest dose (38.12 mg/kg) mean parasite count at this dose was 8±2.73. Even though not comparable to the highest dose, the extract at the lower doses (19.06 and 9.5 mg/kg) was also capable of decreasing (P=0.0002) parasite load to 89±7.41 and 44±7.41, respectively, while mean parasite count in the untreated control group was 190±39.68, the standard drug (Cofantrine 10 mg/kg) parasite count was respectively 146±17.10 to 159±32.86 at day 8pi to 254±6.51 to 159±32.86 (Figure 4a). These data indicate that prophylactic treatment of mice with *A. schweinfurthii* can prevent and significantly reduce proliferation of parasites in the ECM model.

3.7. Effect of Extract on Rectal Temperature in Prophylactic Test

Rectal temperature was <36°C for all groups of mice on the day of PbA infection (Day 0). *A. schweinfurthii* extract-treated mice at lowest doses of 3.8 mg/kg preserved (P=0.0015) reduction in rectal temperature compared to control infected and treat with distilled water. This effect was much higher (P < 0.001) in mice treated with the standard drug Cofantrine failed in prevention temperature loss compared to CINT group at day 4 to 10 pi while group to Asp 38.12, 19.06 and 9.5 presented greater value of rectal temperature was recorded from same day but did not show significant difference (P=0.8915) (Figure 4b).
3.8. Asparagus Schweinfurthii Attenuated Brain Inflammation in PbA-infected Mice

Histopathological studies in rescue therapy on mice infested with the parasite showed the presence of inflamed cells (Figure 5). The group administered aqueous extract of Asparagus schweinfurthii (Figure 5 a, c, d) showed no degenerative changes in the morphology of the brain except group treat which Asp 19.06 (Figure 5b) when compared to negative control.

Figure 5. Photomicrograph of Brain of mice (a) mice infected treated with Asp 38.12 mg/Kg showing no inflammatory cells (H and E, ×100), (b) mice infected treated with Asp 19.06 mg/Kg showing high inflammatory cells (H and E, ×100), (c) treated with Asp 9.5 mg/Kg showing no inflammatory cells (H and E, ×100), (d) treated with Asp 3.8 mg/Kg showing no inflammatory cells showing few inflammatory cells (H and E, ×160), (e) infected with Plasmodium berghei showing high amounts inflammatory cells (H and E, ×160), and (f) Uninfected showing normal brain without inflammatory cells (H and E, ×100).

Histopathological assessments during prophylaxis test of the brain reveal no cell inflammation in all the treated with A. schweinfurthii except the mice treated with 19.06 mg/kg of aqueous extract of A. schweinfurthii which presented middle cell...
inflammation when it is compared to the normal saline (Figure 6 h). The group treated with Cofantrine presented middle cell inflammation compared to negative control.

4. Discussion

In order to gain information on extract’s toxicity in animals and verify its direct relevance for protecting human or animal health, an acute toxicity test at limit doses of 2000 mg/kg was performed. The results suggested an LD50 dose higher than the limit dose 2000mg/kg classifying our extracts as practically nontoxic and without any risk for human health as reported in the OECD guidelines [22]. This finding is consistent with a reported LD50 oral dose of aqueous extract of *A. schweinfurthii* which indicated of higher than 2000 mg/kg confirming the clinical safety of the plant [25].

SHIRPA can evaluate the related behavior of mice to reflect the real-time function of central nervous system [23, 24], it can be used to objectively evaluate the disease process of mice and provide a tool for evaluating new adjuvant therapies. During the observation period, ECM mice gradually showed signs of walking instability, ataxia, and disappearance of auricle reflex, convulsions, coma symptoms and death. The results of SHIRPA score were consistent with those of mice. In this study, significant (P < 0.001) increase in the SHIRPA scores of ECM mice was observed in CINT group. Moreover, the *A. schweinfurthii* also significantly improved the SHIRPA score of mice at d 8, 10, 11 p.i.; as compared CINT exerted greater effects on improving and health behavior of ECM mice. Based on the ECM-specific neurological and behavioral evaluation, we confirmed that aqueous extract of *A. schweinfurthii* prevented significant deterioration of neurological functions.

Clinical symptom such as hypothermia was monitored. Rectal thermometry is a common method of measuring body temperature in rodents to control energy balance or energy metabolism in vivo. Body temperature reductions is common characteristics of *P. berghei* infected mice [26]. A decrease in the metabolic rate of infected mice occurs before death and is accompanied by a corresponding decrease in internal body temperature [27]. So, ideal antimalarial agents obtained from plants are expected to prevent these decreases in infected mice due to the rise in parasitemia. Knowing that the normal temperature of mice varies between 36.5°C and 38°C, the aqueous extract of *A. schweinfurthii* induced body temperature drop on day 4 to day 6 compared to normal control but also prevented the temperature decrease due to infection compared to negative controls. The same observation was previously reported and confirmed for Plasmodium infected mice [28-30].

In vivo, mice survived until day12 pi, the limit for neurological symptoms in this model. Anecdotal information ascribes antimalarial activity to *A. schweinfurthii* [31, 32] but there are no reports of chemoprophylaxis or chemotherapeutic effects of *A. schweinfurthii* on cerebral malaria. There is a close resemblance in malaria pathophysiology between *P. berghei* and *P. falciparum*, the virulent species of human malaria parasites, warranting former to be used as a safer analogue in experimental malaria. Studies indicated that *P. berghei* causes Severe malaria (SM) proceeding to cerebral malaria (CM) in younger animals [33]. Here, demonstrated is the novel influence of pre-infection po administration of *A. schweinfurthii* on malaria in young, six weeks old mice (15-18g) displaying hyperparasitaemia, clinicals signs of experimental cerebral malaria and temperature loss during *P. berghei* infection.

During malaria, the first immune responses (inflammatory) are induced by monocytes to control parasite multiplication. However, excessive and inappropriate activation of the
immune system can induce to the severe form or lead to death [34]. Mechanisms leading to ECM involve the recruitment of inflammatory cells to the brain, particularly known to be responsible for lethal neuropathology [35]. Histopathological analysis of brain show the presence of inflammatory cells in the parasitized/untreated group as well as in the group treated with Asp 19. 06. The presence of these inflammation could be a marker for degenerative process of the organ made by parasites. This is also evidenced in the significant increase cells inflammation in the group (CINT) treated with distilled water when compared to the normal control. The absence of these inflammatory cells in the group administered Asp 38. 12, 9.5 and 3.8 suggest the ability of antimalarial to restore the normal morphology of the brain. In the ECM model, treatment with *A. schweinfurthii* similarly to cofantrine, greatly restrained the recruitment of cells inflammation in mouse brains after 18 days Pi. It seems logical since at day 12, the parasitemia of the treated mice was very low and the parasite undetectable while it is higher in untreated infected control (CINT). Our results therefore demonstrated that *A. schweinfurthii* may be an effective therapy to suppress neuro-inflammation and improve the efficacy of antimalarial drug treatment and improve the

5. Conclusions

The main objective of this study was to evaluate in mice the effects of aqueous extract of *A. schweinfurthii* on cerebral malaria induced by *P. berghei* both in rescues to prophylactic test. The results of this study provide evidence that, during rescues therapy diverse groups of animals treated with the aqueous extract of *A. schweinfurthii* seeds averted inflammation of brain (except those treated by Asp 19.06) and severe malaria development where SHIRPA score was significantly less P ≤0.001 than group infected and treated by distilled water. Temperature loss observed during malaria infection was also significantly P ≤ 0.01 preserved in all untreated infected control (CINT). Our results therefore demonstrated that *A. schweinfurthii* may be an effective therapy for preventing murine cerebral malaria.

Conflicts of Interest

The authors do not have any possible conflicts of interest.

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