Artemisia vulgaris L. ethanolic leaf extract reverses thrombocytopenia/thrombocytosis and averts end-stage disease of experimental severe *Plasmodium berghei* murine malaria

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ABSTRACT

Background & objectives: Artemisinin isolated from *Artemisia annua* is the most potent antimalarial against chloroquine resistant *Plasmodium falciparum* malaria. We previously reported that the ethanolic leaf extract of *Artemisia vulgaris,* an invasive weed and the only *Artemisia* species in Sri Lanka, possess both potent and safe antimalarial activity (in terms of antiparasitic properties) in a *P. berghei* murine malaria model. We report here a prototype study that investigated antidisease activities of *A. vulgaris* ethanolic leaf extract (AVELE) in a *P. berghei* ANKA murine malaria model that elicit pathogenesis similar to falciparum malaria. Profound thrombocytosis and thrombocytopenia in mice were detected in early-stage (Day 3), and at a later stage of infection (Day 6), respectively. *Plasmodium berghei* infected mice, 7 or 8 days post-infection reached end-stage disease with rapid drop in body temperature and usually die within 24 h, as a consequence of cerebral malaria.

Methods: Three doses of the AVELE (500, 750 and 1000 mg/kg) were used to assess antidisease activity of *A. vulgaris* in terms of survival, effects on thrombocyte related pathology and end-stage disease, antipyretic activity, and antinociception, using standard methodology.

Results: The 1000 mg/kg dose of AVELE significantly increased survival, reversed the profound thrombocytopenia/thrombocytosis ($p \leq 0.01$), altered the end-stage disease ($p \leq 0.05$), and manifested significant antipyretic and antinociceptive ($p \leq 0.05$) activities.

Interpretation & conclusion: We conclude that a crude ethanolic leaf extract of *A. vulgaris*, showed potent antimalarial properties, in terms of antidisease activities; antipyretic activity, peripheral and central antinociception, increased survival, averted end-stage disease and reversed thrombocytopenia/thrombocytosis.

Key words Antidisease activity; *Artemisia vulgaris;* end-stage disease; *Plasmodium berghei* ANKA; Sri Lanka; thrombocytopenia/thrombocytosis reversal

INTRODUCTION

At present, malaria remains one of the greatest challenges of global health. Antimalarial drug resistance is thus identified as one of the major causative phenomena hindering malaria control. Artemisinin, a sesquiterpene lactone compound isolated from the plant *Artemisia annua*[1] containing regimens meet the urgent need of effective treatment for multidrug resistant malaria and are advocated for widespread deployment[2]. Nevertheless, emergence of parasites resistant to artemisinin at the Thai-Cambodia border could seriously undermine the success of global malaria control efforts[3]. At present when the control of malaria is increasingly limited by the growing resistance of the malaria parasites to available drugs, the need for much better use of existing drugs, as well as the development of new antimalarials has become an imperative need.

Anthelminthic, antiseptic, antibacterial and anti-inflammatory properties of plant species of the Genus *Artemisia* (family Asteraceae) is widely studied and reported[4–5]. *Artemisia vulgaris* (English – common wormwood or mugwort; Sinhala – *Walkolondu;* Tamil– *Mâcipattiri*), is an invasive weed, growing on nitrogenous soils, found in waste dumps and on roadsides. It is the only *Artemisia* species prevalent in Sri Lanka. Previous studies by our group substantiated that oral administration of organic and ethanolic extracts of *A. vulgaris* respectively showed moderate and high antiparasitic activity in *Plasmodium yoelli*[6] and in *P. berghei* ANKA[7] rodent malaria models.

In vivo testing of antimalarial activity of developed formulations by using *P. berghei*-infected mice as a suitable model for studying malaria is promising, as the infection presents structural, physiological and life cycle analo-

gies with the human malaria caused by *P. falciparum*[8].

A characteristic feature of malaria is the presence of fever episodes. Rare attempts were made to observe this phenomenon in rodent models. Mice infected with *P. berghei* are considered to be in the end-stage disease when their body temperature rapidly drops ($\leq 35.5°C/ 95°F$), 7 or 8 days post-infection and usually succumb to the infection within 24 h, as a consequence of cerebral malaria[9]. Though such observations are scarcely documented for rodent malaria, it is a well-established fact associated with other fatal diseases such as renal[10] and cardiac failure[11]. End-stage disease is the manifestation of the worst condition for an organ or disease state. At this point the organ barely functions.

Several studies report that platelets may play a key role in the immune-pathophysiology of experimental, severe *P. berghei* malaria[12–13] as well as of falciparum malaria[14]. In childhood falciparum malaria, median platelet counts were lower among severe cases than in mild cases, and in children who died than among those who recovered. Moreover, multivariate analysis identified thrombocytopenia as an independent predictor of death associated with malaria[15]. Interestingly, some studies indicate a protective function for platelets in the early stages of erythrocytic infection in malaria which demonstrated that purified human platelets killed *P. falciparum* parasites cultured in red blood cells and increase production in early stages of the infection in *in vivo* models[16]. It was later established that platelet factor 4 and the erythrocyte Duffy antigen receptor were required for platelet killing of *P. falciparum*[17].

Elevated body temperature[18], body aches and headaches[19] are classic malaria symptoms. Therefore, patient management is one of the common practices associated with malaria treatment. Thus, a plant extract manifesting a plethora of antimalarial disease activities will prove to be very useful to reduce disease complications.

Thus, we undertook a study to investigate antidisease properties of *A. vulgaris* ethanolic leaf extract (AVELE) in the *P. berghei* ANKA (lethal strain) murine malaria model that elicit pathogenesis similar to falciparum malaria. *In vivo* antidisease activity of AVELE was investigated using standard methodology. Especially, effects of AVELE on malaria induced critical pathologies such as thrombocytopenia/thrombocytosis and the end-stage disease were examined for the first time.

MATERIAL & METHODS

Ethical approval
Ethical clearance for this research study was obtained from the Ethics Review Committee of the Faculty of Medicine, University of Colombo, Sri Lanka (Ref No. EC-10-132).

Collection and authentication of plant material
Leaves of *A. vulgaris*, were collected from the roadsides in Nuwara Eliya (altitude: 1868 m, 6.9667° N, 80.7667° E), Sri Lanka. The specimen were authenticated by the Herbal Technology Division, Industrial Technology Institute, Sri Lanka, and a voucher specimen was deposited in the museum of the Department of Zoology, Faculty of Science, University of Colombo, Sri Lanka (GY/01/2010).

Preparation of the plant extract
The *A. vulgaris* ethanolic leaf extract (AVELE) was prepared essentially following the procedure described by Bamunuarachchi *et al*[7]. Briefly, washed, air-dried leaves of *A. vulgaris* were powdered and soaked in an organic solvent mixture consisting of diethyl ether, methanol and petroleum ether at a 1:1:1 ratio. The resulting dark green solution was filtered and the leaves were soaked in the organic solvent mixture for a second time. The extracted solution was then evaporated at reduced pressure using a rotavapour. This crude extract was suspended in 5% ethanol. The test animals were orally treated with the AVELE at the doses of 250, 500, 750 and 1000 mg/kg of body weight that represented low, human equivalent, moderate high and high doses, respectively[7].

Experimental animals
Healthy inbred adult male ICR (Institute of Cancer Research) mice weighing 25–30 g, purchased from the Medical Research Institute, Colombo, were used in this study. All the animals were housed in plastic cages in the animal house, Department of Zoology, University of Colombo under standard conditions (temperature 28–31°C, photoperiod: approximately 12 h natural light per day, Relative humidity: 50–55%). The animals were given *ad libitum* access to food pellets (Master Feed Ltd., Colombo, Sri Lanka) and drinking water. Except at the time of experimental procedure, the animals were handled only during cage cleaning.

Parasite isolates
Plasmodium berghei ANKA parasites maintained through serial blood passage in mice were used to assess *in vivo* antimalarial activity of AVELE.

Evaluation of survival for infected mice
A 4-day suppressive assay[20] was used to evaluate the

survival of infected mice (n = 6/group). AVELE (250, 500, 750, 1000 mg/kg) and control (5% ethanol) were administered to all the test animals with prior exposure to *P. berghei* from Day 0 through D3. Mice were observed daily for eight consecutive days and their parasitaemia was monitored[7]. Mice were treated orally with Coartem® when parasitaemia reached 50%. The number of mice that survived in each group with levels of parasitaemia below 50% at the end of eight days was recorded.

Investigation of temperature fluctuations associated with P. berghei rodent malaria

A group (n = 8) of mice was injected intraperitoneally (IP) with 10^7 infected RBC on D0. The rectal temperature of mice was recorded twice a day, once in the morning (0900–1000 hrs) and repeated in the evening (1500–1600 hrs) using a digital thermometer (VT-801 series, Valeo Corporation, Taipei, Taiwan) from D0 through D8. Simultaneously, blood smears were prepared (morning and evening) from tail bleed of mice and parasitaemia levels were determined. Rectal temperature of normal, uninfected mice (n = 8) were also recorded.

Effect of AVELE on the temperature fluctuation of mice infected with P. berghei

Four groups (n = 8/group) of male ICR mice were infected with 10^7 *P. berghei*-infected RBC on D0. Daily oral administration of the AVELE (500, 750, and 1000 mg/kg) and control (5% ethanol) took place from D0 through D3. The rectal temperature of mice was determined from D0 through D8, twice daily, in the morning (0900–1000 hrs) and in the evening (1500–1600 hrs). Concurrently, blood smears were prepared from tail bleed of mice and parasitaemia levels were determined for each group.

Effect of AVELE on the thrombocyte-related pathology associated with P. berghei rodent malaria

Four groups of male ICR mice (n = 6/group) were inoculated with 10^7 *P. berghei*-infected RBC on D0. AVELE (500, 750, and 1000 mg/kg) and control (5% ethanol) were administered to all from D0 through D3, where their thrombocyte counts[21] and parasitaemia were monitored from D0 through D6. Thrombocyte counts of normal, untreated mice (n = 6) were also recorded.

Antipyretic activity of AVELE investigated in the yeast-induced mice pyrexia model

Total 24 mice were randomly assigned into four equal groups (n = 6/group). The rectal temperature of mice were determined using a digital thermometer (VT-801 series, Valeo Corporation, Taipei, Taiwan). Mice were then sub-cutaneously injected with 0.3 ml/kg of 15% (w/v) aqueous suspension of active dry yeast (Instant-Dry yeast, AB Mauri India Pvt. Ltd, Ratnagiri, India) to induce pyrexia. The rectal temperature of these mice was determined 19 hours later[22], and subsequently the four groups were orally treated with AVELE (500 and 1000 mg/kg body weight doses), 5% ethanol (control), and the reference drug, Paracetamol (6 mg/kg), respectively. Subsequently, the rectal temperature of test animals was determined at 1, 2, 3, 4, 5 and 6 h post-treatment.

Investigation of antinociceptive effects of AVELE

Hot plate test and tail flick methods were used, respectively, to assess the central and spinally mediated antinociceptive effects of AVELE[23]. Four groups (n = 6/group) of healthy male ICR mice were used; the first two groups were treated with 500 and 1000 mg/kg doses of AVELE, while the third group received 5% ethanol (test control group), the fourth group treated with distilled water functioned as the normal group.

The reaction times of the mice were measured one hour prior to treatment. In the hot plate test, the mouse was placed inside the hot plate analgesia meter (Model MK 35A, Muromachi Kikai Co. Ltd., Tokyo, Japan) at 52°C and the time taken for animals to lick the hind paw or to jump was recorded. A cut-off time of 20 sec was set to avoid tissue damage. In the tail flick test, 2–3 cm of the tail from the tip was immersed in a hot water bath at 54°C and time taken to flick the tail was recorded. Readings were taken one hour post treatment and then repeated at hourly intervals for 5 h.

Writhing test[24] was used to assess peripheral analgesia using four groups (n = 6/group) of male ICR mice; two groups were treated with either 500 or 1000 mg/kg AVELE, the third group with 10 mg/kg of diclofenac sodium (reference drug) and the control group with 5% ethanol. One hour later, 0.05 ml of 10% acetic acid was injected IP to each of these mice, and the number of writhing (abdominal contractions and stretches) that occurred between 5 and 20 min were recorded. Percentage inhibition of writhing was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{(\text{Mean number of writhing in control group} - \text{Mean number of writhing in test group})}{\text{Mean number of writhing in control}} \times 100$$

Statistical analysis

Data were analyzed using the Minitab 15 statistical package for Windows. Data were expressed as mean ± SEM. Statistical analysis was performed using the Mann-Whitney U-test. Significance was set at $p \leq 0.05$.

RESULTS

Survival of test animals

Figure 1 illustrates the effect of oral administration of AVELE on the survival of mice infected with *P. berghei*. The group treated with the high dose (1000 mg/kg) of the extract and the positive control group showed maximum (100%) survival.

Effect of AVELE on rectal temperature fluctuation of P. berghei-infected mice

Body temperature associated with *P. berghei* infected mice showed that within 6 h (morning compared with evening), their rectal temperature fluctuated by 3°F in the control group, D1 through D6 which was a significant manifestation ($p \leq 0.01$) absent in the uninfected group (Fig. 2a). Importantly, mice treated with 500, 750 and 1000 mg/kg doses of the AVELE significantly ($p \leq 0.01$) deviated from this and maintained normal
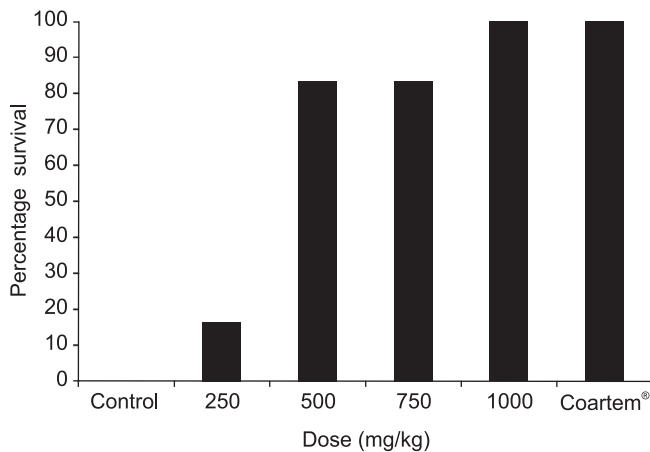

Fig. 1: Effect of oral administration of AVELE on survival of mice infected with *P. berghei* in the 4-day suppressive assay (n = 6/group).
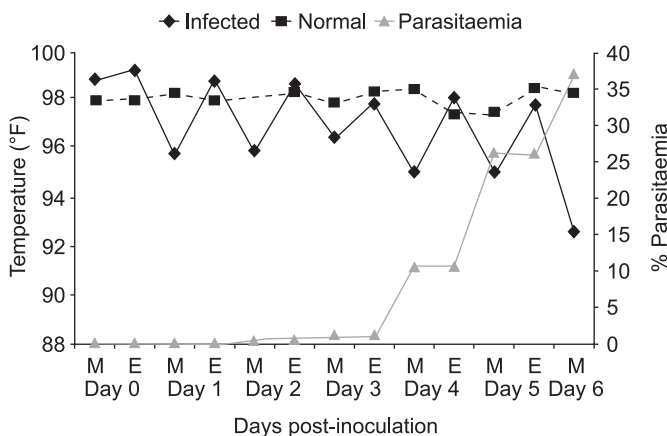

Fig. 2a: Temperature fluctuations associated with *P. berghei* rodent malaria model (M: Morning, E: Evening).
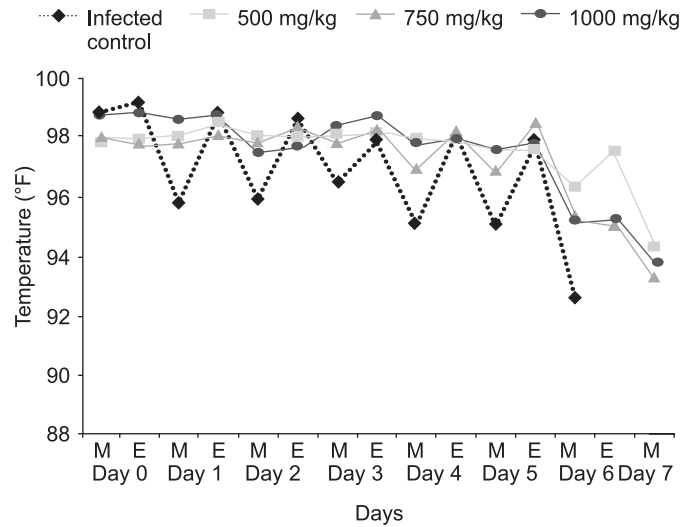

Fig. 2b: Effect of AVELE against the temperature fluctuation associated with *P. berghei* mouse model (M: Morning, E: Evening).

rectal temperatures until Days 5 and 6 post-inoculation (Fig. 2b).

Effect of AVELE on P. berghei malaria-associated thrombocytosis/thrombocytopenia in mice

On D3, at early-stage of disease establishment, profound thrombocytosis in the control was observed compared to the healthy, untreated group ($p \leq 0.01$) (Fig. 3a). Importantly, 500, 750 and 1000 mg/kg doses of AVELE significantly ($p \leq 0.01$) reversed this condition by 27.5, 47.3 and 41.5 %, respectively, compared with the control (Table 1 and Fig. 3b).

On D6, at a much later stage of the infection, significantly ($p \leq 0.01$) profound thrombocytopenia was detected in the control compared to the healthy, untreated group (Fig. 3a) which was observed to be significantly ($p \leq 0.01$) reversed by the 500, 750 and 1000 mg/kg doses of
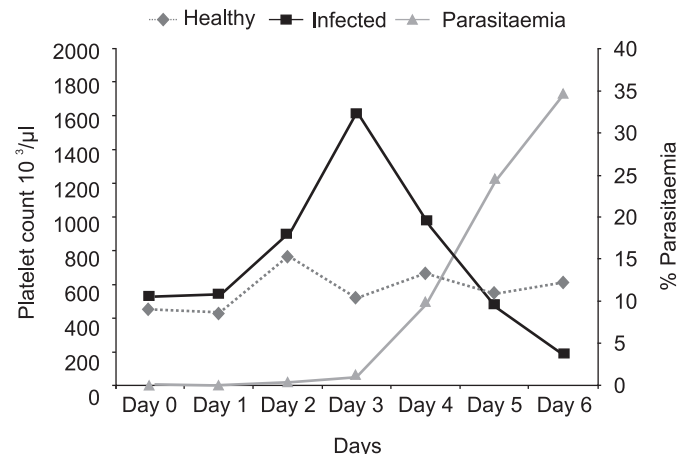

Fig. 3a: Platelet count fluctuations associated with *P. berghei* rodent malaria model.

Table 1. Effect of AVELE on thrombocyte-related pathology associated with *P. berghei* rodent malaria

Treatment	Platelet count × 10³/µl						
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Healthy	484.2±22.1	458.5±34.9	817±45.2	556±46.8	712±55.1	583.8±22.3	655±11.9
Control (5% EtoH)	564.8±48.3	578.3±52.5	957.7±86.9	1724±128[#]	1041.2±78.6	517.2±44.4	197.8±26.7[#]
500 mg/kg	531±27.7	518.3±12.8	946.8±68.6	1249.3±18[†]	1223±54.9	1193.2±89.8[†]	1167±175[†]
750 mg/kg	464.8±38.8	437±35.2	479.2±35	908.3±74[†]	1135±63.7	1773±113[†]	2023±119[†]
1000 mg/kg	320.7±58.6	333.5±55.6*	355.8±29.9	1007±42.8[†]	1040±102	904±146*	840±107[†]

Values are expressed as means±SEM (n = 8); [#]$p < 0.01$ as compared with the healthy, untreated group; *$p < 0.05$ and [†]$p < 0.01$, as compared with the control (Mann-Whitney U-test).
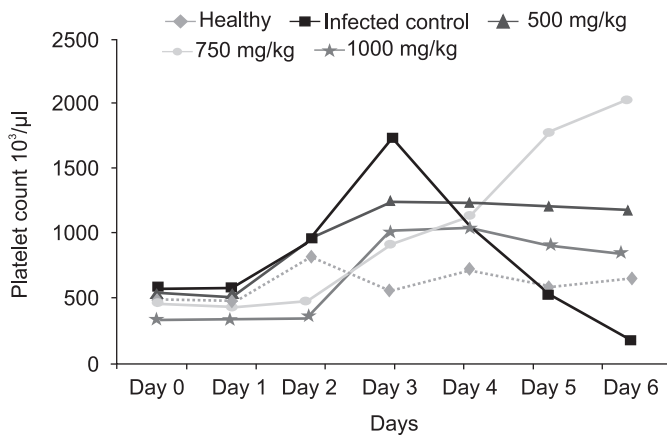

Fig. 3b: Effect of AVELE on mouse platelet count fluctuations associated with *P. berghei* rodent malaria.

AVELE, by 489.9, 922.7 and 324.6%, respectively (Table 1 and Fig. 3b).

Antipyretic activity of AVELE in the yeast-induced mice pyrexia model

AVELE at 500 mg/kg dosage significantly ($p < 0.05$) suppressed the yeast induced pyrexia (>100°F) in the fourth hour which significantly lasted for only an hour. Conversely the 1000 mg/kg dose of AVELE significantly ($p < 0.05$) suppressed pyrexia from the second hour onwards that was sustained until fourth hour (Table 2).

Evaluation of antinociceptive activity of AVELE

When compared with the control, mice treated with the 1000 mg/kg dose of AVELE showed significantly

Table 2. Effect of oral administration of AVELE (500, 1000 mg/kg), and paracetamol on yeast-induced pyrexia in mice

Treatment	Rectal temperature (°F)							
	Before yeast-pyrexia	After induced	Post-treatment					
			1st hour	2nd hour	3rd hour	4th hour	5th hour	6th hour
Control (5% EtoH)	98.16±0.56	100.13±0.54	99.05±0.34	99.58±0.45	100.00±0.45	100.18±0.35	100.22±0.31	99.96±0.22
AVELE 500 mg/kg	98.08±0.34	99.63±0.37	98.16±0.52	98.33±0.42	98.96±0.33	98.46±0.34*	99.25±0.36	99.71±0.25
1000 mg/kg	98.35±0.40	100.15±0.37	97.61±0.60	97.36±0.59*	97.92±0.59[†]	98.27±0.34*	99.25±0.63	99.41±0.63
Paracetamol (6 mg/kg)	97.9±0.52	99.65±0.54	95.70±0.16[†]	96.2±0.24[†]	96.61±0.41[†]	96.96±0.38[†]	97.35±0.43[†]	97.85±0.44[†]

Values are expressed as means ± SEM (n = 8); *$p < 0.05$ and [†]$p < 0.01$, as compared with the control (Mann-Whitney U-test); Ambient temperature = 84°F.

Table 3. Reaction times of mice in the hot plate test following oral administration of AVELE
(500, 1000 mg/kg doses), distilled water and control (5% ethanol)

Experimental group	Reaction times for hot plate test (sec)					
	Initial	1st hour	2nd hour	3rd hour	4th hour	5th hour
Normal group (DW)	6.37±0.48	5.98±0.56	6.13±0.75	6.77±0.79	6.02±0.65	5.91±0.59
Control group (5% EtoH)	7.16±0.52	6.50±0.66	6.02±0.69	6.03±0.78	6.71±0.35	6.32±0.35
Test group (500 mg/kg AVELE)	6.36±0.55	7.72±0.54	6.23±0.53	4.83±0.45	4.38±0.47	5.41±0.41
Test group (1000 mg/kg AVELE)	6.88±0.30	6.36±0.81	6.08±0.48	6.35±0.75	8.85±0.57[†]	8.86±0.58[†]

Values are expressed as means ± SEM (n = 6; [†]$p \leq 0.01$ as compared with the control (Mann-Whitney U-test).

Table 4. Analgesic activity of AVELE in acetic acid induced writhing response in mice

Treatment	No. of writhing/ unit time	Percentage inhibition
Control (5% EtoH)	25.57±1.29	–
AVELE		
500 mg/kg	16.86±1.34*	34.1
1000 mg/kg	13.57±0.86*	46.9
Diclofenac sodium (10 mg/kg)	13.28±0.68*	48.1

Values are expressed as means ± SEM (n = 6/group); *$p \leq 0.01$ as compared with the control (Mann-Whitney U-test).

($p \leq 0.01$) prolonged reaction times (fourth hour by 31.9%; fifth hour by 40.1%) in the hot plate test but not in the tail flick test (Table 3), indicative of the ability of AVELE to curb acute pain, central supra spinally.

The assessment of peripheral analgesic effect of both 500 and 1000 mg/kg doses of AVELE, resulted in significantly ($p \leq 0.01$) reduced acetic acid induced writhing reaction in mice, the latter which in effect was comparable to that of the reference drug, Diclofenac sodium that act on sustained pain (Table 4).

DISCUSSION

Currently, the first line chemotherapeutics against malaria are fixed dose Artemisinin-based combination therapies (ACTs), which are presumed to act against the blood stages of all human malaria[25]. However, emergence of parasites resistant to artemisinin at the Thai-Cambodia border[3] would seriously undermine the effective use of ACTs. Thus, the relentless pursuit of novel antimalarials is a worthy exercise.

Artemisia vulgaris is not commonly used to treat malaria but is reported to possess many other ethnopharmaceutical properties[4–5]. Our group previously substantiated safe and highly potent antiparasitic activity of *A. vulgaris* ethanolic leaf extract when administered orally, against *P. berghei* ANKA rodent malaria parasites[7].

The rodent malaria model used in this study, the lethal strain of *P. berghei* ANKA, as mentioned before, causes severe malaria in mice and the infection elicits structural, physiological and life-cycle analogies with the human disease[8]. These parasites elicit experimental severe malaria (ESM), and comparatively similar pathogenesis characters with *P. falciparum* human malaria, where infected mice exhibit a marked systemic inflammatory response, profound thrombocytopenia, and cerebral malaria[12]. *Plasmodium berghei* ANKA infection in inbred ICR mice as a model of cerebral malaria (CM) can reproduce many of the important features of CM and, therefore, can be used

as a tool to advance our understanding of the disease pathogenesis [26–27]. Cerebral malaria, the major severe form of the infection, is lethal and in humans results from *P. falciparum* infections. Therefore, any potential antidisease activity of the AVELE manifested in the *P. berghei in vivo* model may be presumed as potential activity against pathological complications of *P. falciparum* malaria, though such interpretation should be made with caution.

Patient management is one of the common practices associated with malaria treatment for the reason that malaria depicts several disease complications such as cerebral malaria, profound thrombocytosis and thrombocytopenia, fever episodes, elevated body temperature and body aches and headaches[12, 14–16, 19]

In human malaria pathology, a characteristic feature is the presence of fever episodes. Rare attempts have been made to observe this phenomenon in murine models. Mice infected with *P. berghei* parasites exhibit a rapidly decreasing body temperature and these succumb to the disease within 24 h, 8 or 9 days into the infection. Mice are considered to be in end-stage disease as a consequence of CM[9]. We too observed this phenomenon of end-stage disease in mice infected with *P. berghei* but importantly, those test animals treated with 500, 750 and 1000 mg/kg doses of AVELE significantly ($p \leq 0.01$) deviated from this debacle and clearly maintained normal rectal temperature until 5 and 6 days post-inoculation. Thus, it may be a clear indication that AVELE alleviate the cerebral complications associated with rodent malaria.

One of the hallmarks of CM, caused by infection with *P. falciparum*, is petechial haemorrhaging in the brain, indicating that platelets may play a crucial role in malarial pathogenesis[28]. Both thrombocytopenia and platelet-induced clumping of *P. falciparum*-parasitized RBCs is a common feature of acute malaria and occurs in both *P. falciparum* and *P. vivax* infections regardless of the severity of infection[14]. The current study for the first time demonstrated that the oral administration of crude AVELE significantly reversed both thrombocytosis and thrombocytopenia that occurred in *P. berghei* murine malaria, which reiterated that AVELE may have the potential to lessen cerebral complications and haemorrhages associated with CM.

At least three different types of antimalarial activity can be evaluated. First, activity is considered to be *prophylactic* if the action is exerted against the sporozoites or the parasites of the initial tissue phase of the disease; *suppressive*, if against the parasites of the asexual erythrocytic phase of the disease; and *curative*, if against the parasites of the persisting tissue phase[2]. The most widely used initial test for *in vivo* screening of antimalarial com-

pounds, which uses *P. berghei* or less frequently *P. chabaudi*, is a 4-day suppressive assay[29]. We previously demonstrated that the 1000 mg/kg dose of the AVELE possess antiparasitic activity in the 4-day suppressive assay but not in the curative assay, which suggests that this crude plant extract in this study manifested suppressive but not curative activity[7]. Thus, it may be presumed that the concentration of the active compound in the crude extract may not have sufficed to eliminate an established infection. This highlights the need for future *in vitro* and *in vivo* investigations using the isolated active component(s) of *A. vulgaris* to substantiate its potential as a therapeutic agent against malaria and also to elucidate its mode of action.

Furthermore, the 1000 mg/kg dose of *A. vulgaris* leaf extract exhibited significant antipyretic activity in the yeast-induced mice pyrexia model. Elevated body temperature is a classic malaria symptom. As the malaria parasites enter the blood stream they infect and destroy red blood cells. Destruction of these essential cells lead to fever[12]. Therefore, fever management is one of the common practices associated with malaria treatment. Despite widespread use of Paracetamol in fever management, a study on humans infected with malaria concluded that Paracetamol can prolong malaria parasitaemia[30]. Thus, the anti pyretic property of *A. vulgaris* crude plant extract in concert with established antimalarial properties, further corroborates the potential of this plant to be developed in to an effective antimalarial.

Supra spinally mediated acute pain killing effect of an organic extract of *A. vulgaris* leaves was previously reported by us in a *P. yoelli* mouse model[6]. More importantly, when we evaluated the antinociceptive potential of AVELE, acute (supra spinally mediated) as well as peripheral analgesic effects were manifested where the effect of the high dose of AVELE was comparable to that of the standard peripheral analgesic drug, Diclofenac sodium. The peripheral analgesic effects were monitored using acetic acid induced writhing of mice[24]. The quantification of prostaglandins by radio immuno assay in the peritoneal exudates of rats, obtained after intraperitoneal injection of acetic acid, found high levels of prostaglandins, PGE2 and PGF2 alpha during the first 30 min after acetic acid injection[31]. Nevertheless, it was also recorded that the intraperitoneal administration of acetic acid induces the liberation of not only of prostaglandins but also of the sympathetic nervous system mediators[32]. Thus, it may be assumed that *A. vulgaris* ethanolic leaf extract may significantly act either by inhibiting the action of prostaglandin enzyme, prostaglandin E synthases, or by blocking the prostaglandin receptor. It may also act on the sympathetic nervous system mediators. As malaria elicits body aches and pain, this effective systemic antinociceptive (peripheral pain killing) property of the AVELE, that reiterates the findings of a study by Pires *et al*[23], will be exceptionally potent in the treatment of malaria. It may also be presumed that the responsible active ingredients of the crude plant extract may form the basis for an excellent novel analgesic.

Whether for chemotherapy or chemoprophylaxis, toxicity of drugs prescribed for malaria is a closely monitored factor. Toxicity has been singled out as the main drawback of traditional herbal antimalarial preparations[6]. Thus, we previously evaluated 30 days chronic oral administration of the 1000 mg/kg dose of AVELE, which showed no overt signs of toxicity or stress. Hepatotoxicity (evaluated in terms of serum GOT and GPT levels), renotoxicity (in terms of serum urea and creatinine) and haematotoxicity (in terms of RBC, WBC and DC counts) were also ruled out. Weight and gross morphology of vital organs (liver, spleen, kidneys, heart, and lungs) were also not affected. Also continuous weight gain of animals was observed. Thus, the *A. vulgaris* extract was well-tolerated by mice[7].

Our previous study demonstrated that the oral administration of a crude leaf extract of *A. vulgaris*, possess safe and potent (87.3% parasitaemia inhibition) antimalarial effects, in terms of antiparasitic activity in a *P. berghei* ANKA lethal murine malaria model[7]. In conclusion, this study for the first time demonstrated that the oral administration of a crude leaf extract of *A. vulgaris*, possesses potent antidisease action (antipyretic activity, peripheral and central antinociception, and reversal of thrombocytopenia/thrombocytosis and of end-stage disease), in a *P. berghei* murine malaria model. Thus, the antidisease properties of *A. vulgaris* crude plant extract collectively with established antiparasitic properties, further corroborate the potential of this plant to be developed into an effective antimalarial.

Activity directed fractionation and further investigations on antiparasitic and antidisease activity of the purified components may hopefully lead to significant scientific drug discovery. More importantly, *A. vulgaris* is a weed distributed in the hilly parts of Sri Lanka in high abundance, and thus has the potential to be developed into a cheap source of plant-based antimalarial in future.

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