Chemotherapeutic Interaction of *Vernonia amygdalina* (delile) Leaf Extract with Artesunate and Amodiaquine in Murine Malaria Model

ABSTRACT

Aim of the study: Conventional antimalarial drugs are used concurrently with herbal remedies **i**n malarial endemic developing countries. *Vernonia amygdalina* is one of such popular herbs used in the treatment of malaria. This study aimed at investigating the antimalarial chemotherapeutic interaction of *Vernonia amygdalina (*VA) when combined with Amodiaquine (AQ) and/or Artesunate (AS) in a murine *Plasmodium berghei* malaria model.

Methodology: Various doses of aqueous VA leaf extract (100-500 mg/kg/day), AQ (2-10 mg/kg/day) and AS (0.8-4 mg/kg/day) were administered orally to *P berghei.*-infected Swiss albino mice to determine their sub-therapeutic doses, which were then used to investigate the chemotherapeutic interactions of VA with AQ and/or AS in both early and established malaria infection test models. The survival of animals with established infections that received different drug/herb treatments were determined using their mean survival time (days) and Kaplan-Meier survival curves (percentage). The data obtained were analyzed using GraphPad Instat (version 3.10) and Prism^{R} (version 5.01) and then subjected to One-way ANOVA, followed by Student-Newman-Keuls test, where $P < .05$ was considered statistically significant.

Results: The sub-therapeutic doses of VA, AQ and AS were found to be 100 mg/kg, 2 mg/kg and 2.4 mg/kg respectively. The chemosuppressive effect of AQ or AS was significantly increased ($p< 0.05$) when administered in combination with the VA extract. Similarly, combination of VA extract with AQ or AS resulted in significant ($P < .05$) parasite clearance when compared to the effects of the herb or the conventional drugs administered separately. The mean survival period of animals with established infection was also significantly enhanced by the VA alone or with AQ(or AS) compared to placebo.

Keywords: Chemotherapeutic Interaction; Murine Malaria Model; *Vernonia amygdalina,*; Artesunate;

1. INTRODUCTION

From the World Malaria Report 2018, malaria prevalence is most documented in tropical countries involving over 219 million cases and 435,000 estimated deaths, with high vulnerability being among children under five and pregnant women [1]. The sub-Saharan African Region bears the bulk of morbidity and mortality from malaria, with West African countries accounting for a larger percentage of malaria cases and deaths [1]. The advocacy of Artemisinin-Based Combination Therapies (ACTs) such as artesunate-amodiaquine, as the standard practice in malaria chemotherapy, has globally reduced the estimates of malaria cases and deaths [2]. However, recently, the detection of *P. falciparum* resistance to artemisinin in Southeast Asia, is threatening the gains recorded in combating malaria [1]. This worrisome

emergence of *P. falciparum* resistance at the Cambodia-Thailand border has raised the possibility that malaria parasites strains that are resistant to ACTs and other combination therapies may have evolved [3]. The reported incidence of decrease in malaria parasite sensitivity to the ACT drugs is of great concern and this could be devastating for malaria global control. Thus, the need to explore novel antimalarial drugs has become a necessity due to the alarming rate at which *P. falciparum* has developed resistance to old antimalarial drugs as well as other newer synthetic antimalarial drugs [4].

Historically, plants as major source of drugs have produced two important antimalarial drugs, namely quinine and artemisinin, both of which are chemical leads to several synthetic antimalarial drugs [5]. Herbal products are increasingly being employed as a class of agents used in malaria chemotherapy. The availability, accessibility and affordability of several herbal antimalarial products, have led to their wide usage to treat malaria patients, although several factors such as clinical efficacy and safety, preparations, dosages and consensus among traditional healers are still not fully documented [6]. *Vernonia amygdalina* (Delile), (commonly called bitter leaf) belongs to the Asteraceae family and it grows throughout tropical Africa [7]. The antimalarial effects of *Vernonia amygdalina* have been extensively reported by several authors [8, 9, 10, 11]. *Vernonia amygdalina* was also listed as one of the herbal antimalarial with proven efficacy in an overview of herbal antimalarial products that have undergone some form of clinical trials [12].

In malaria therapy, the co-administration of orthodox medicine with herbs has become a common practice particularly in endemic regions which is usually accompanied by a variety of therapeutic implications [11]. A prevalence study carried out in Nigeria, revealed that this practice of concurrent use of conventional antimalarial drugs with antimalarial herbal therapies was not only done by the poor and illiterate but also by the rich and highly educated people [13]. Additive, synergistic, and/or antagonistic pharmacodynamic/pharmacokinetic interactions may result from co-administration of herbal products with conventional drugs. It is therefore important to evaluate the interaction between common herbs used in the management of malaria and some of the conventional antimalarial drugs. Given the facts that *Vernonia amygdalina* is a commonly used antimalarial herb with a verified and reported clinical efficacy, it was found necessary to investigate the therapeutic outcome of concurrent administration of the herb with some commonly used orthodox antimalarial drugs. The present study, therefore, evaluated the in vivo antimalarial activities of aqueous leaf extract of *Vernonia amygdalina* alone and in combination with amodiaquine and/or artesunate in a murine *Plasmodium berghei* malaria model

2. MATERIAL AND METHODS

2.1 Experimental Animals and Parasite

Animals used in this study were adult male and female wistar mice (18-22 g) obtained from the animal house, Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Nigeria. The animals were kept in cages (MEDIWISE, India), housed in a well-ventilated animal house and were allowed to acclimatize for 7 days prior to the commencement of the experiment. They were allowed access to commercial food pellets (Premier Feed Mills, Nigeria) and water *ad libitum* throughout the duration of the experiment. The “Principle of Laboratory Animal Care” (NIH publication No 85-23)

guidelines and procedures were followed in the study [14]. Donor mice infected with chloroquine-sensitive (*Plasmodium Berghei* NK65) parasite obtained from the Institute of Advanced Medical Research and Training (IAMRAT), University of Ibadan, Nigeria, were allowed to develop parasitaemia. The presence of parasitemia was established by microscopic examination of a thin blood film. Each Experimental mouse was then injected with infected blood suspension (0.2 ml) containing about $1x10^7$ suspension of *P. berghei* parasitised red blood cells.

2.2 Drugs

Camoquin plus® (Amodiaquine Suspension and Artesunate Powder), Pfizer, Nigeria.

2.3 Collection, Preparation and Extraction of *Vernonia amygdalina* Leaf

Fresh leaves of *V. amygdalina* (Asteraceae) were collected, botanically identified and authenticated in the Department of Pharmacognosy, Faculty of Pharmacy of Obafemi Awolowo University, Nigeria, by Mr. Ogunlowo. The voucher specimen with the number FPI 2229 (FPI included in the online edition of *Index Herbariorum* Obafemi Awolowo University, Nigeria) was deposited in the herbarium of the same Department of Pharmacognosy of the University. The leaves were air dried for seven days and pulverised, after which five hundred grams (500 g) of the powder was macerated in 2 L of distilled water for 72 hours. The filtrate was freeze-dried using a freeze dryer (Edward Pirani 10 lyophiliser, UK) for 3 days and the freeze dried extract was stored in sterile bottles at 4^0C. This was subsequently reconstituted with normal saline to designated doses before use.

2.4 Determination of Sub-therapeutic Doses of *V. Amygdalina*, Amodiaquine and Artesunate on Parasite Density

The four-day Peter's chemosuppressive test, as modified by Anagu et al. [15] was employed. Briefly stated, all the mice tested received standard inoculum sizes of 1×10^7 chloroquine-sensitive *Plasmodium berghei* infected erythrocytes by intraperitoneal (I.P) route at the commencement of the experiment. Single oral doses of the test drugs or extract were administered to the animals 3 hr after each inoculum for four consecutive days. A range of five oral doses of *V. amygdalina* leaf extract (100, 200, 300, 400 and 500 mg/kg/day), Amodiaquine suspension (2, 4, 6, 8 and 10 mg/kg/day) and Artesunate solution (0.8, 1.6, 2.4, 3.2 and 4 mg/kg/day) were given separately to fifteen groups (n = 5/group. Five groups for each drug/herb). For the negative control, a separate group (n=5) received normal saline (10 µL/g body weight/day). Thin blood smears were made from the tail of each mouse on days 4 and 7 following drug administration to evaluate the parasite density in each test mouse. The parasite density/µL for each test mouse was estimated by counting 200 white blood cells/µL and the number of parasitised red blood cells against the white blood cells counted was noted and multiplied by the median standard total white blood cells count in mice (6500) [16].

The calculation of the parasite densities was done using the equation (1) below:

$$\text{Parasite density /µL} = \frac{\text{Parasite counted}}{\text{WBCs counted}} \times 6500 \qquad (1)$$

Average Percentage Chemosuppression was calculated using the equation (2) below:

$$\frac{A - B}{A} \times 100 \qquad (2)$$

Where A is the Average Parasite Density of the Negative Control Group; B is the Average Parasite Density of the Test Group.

The minimum doses of *V. amygdalina* leaf extracts, artesunate and amodiaquine that caused at least a 30% reduction in parasite density on days 4 post-infection were regarded as the sub-therapeutic doses.

2.5 Assessment of Effects of *V. amygdalina extract* and its Combination with Artesunate and/or Amodiaquine on parasite density in Early Infection

The determined sub-therapeutic doses were combined to assess their antimalarial effects on parasite density using the four-day Peter's chemosuppressive test as earlier described. Infected mice were separated into eight groups (n=5/group) and treatments were administered as follows;

Groups I, II and III: Received the sub-therapeutic doses of *V. amygdalina* (VA), amodiaquine (AQ) and artesunate (AS) respectively;

Group IV: Received the sub-therapeutic dose of *V. amygdalina* (VA) in combination with the sub-therapeutic dose of AQ;

Group V: The sub-therapeutic dose of *V. amygdalina* (VA) was combined with sub-therapeutic dose of AS;

Group VI: The sub-therapeutic dose of *V. amygdalina* (VA) in combination with sub-therapeutic doses of AQ+AS;

Group VII: Sub-therapeutic doses of AQ+AS only;

Group VIII: Received normal saline which served as the negative control group.

Parasite densities and average percentage chemosuppression were calculated.

2.6 Assessment of Effects of *V. amygdalina* and its Combination with Artesunate and/or Amodiaquine on parasite density in Established Infection

The Curative test was carried out according to the method described by Ryley and Peters [17]. Each mouse was inoculated with $1x10^7$ *Plasmodium berghei* infected erythrocytes on the first day of the experiment (Day 1). The mice were not treated until the parasitaemia was developed and established. On day 4 (72 hr after inoculation), the mice were treated orally with the extract and the drug, singly and in combinations. All the animals were treated daily for five days. Eight groups (n=5) of infected mice, as described for the early malaria infection test were also used. Thin blood smears from each mouse tail were made daily for 5 days (D4-D8) post-infection to monitor the parasite density of each test mouse which was then calculated using the equation described earlier.

The average percentage curative effects of each treatment group was calculated using the equation (3) below.

$$\frac{A - B}{A} \times 100 \qquad (3)$$

Where A is the Average Parasite density of the negative control group; B is the average Parasite density of the test group.

2.7 Survival Period Determination

After the five days of drug and/or herbal extract administrations, the animals were monitored for 28 days and the number of deaths and survival for each group during this period were recorded. The mean survival time for each group was determined. Also, Kaplan-Meier survival curves were plotted in order to estimate percentage survival of the animals for each group.

2.8 Statistical Analysis

The data were analysed using GraphPad Instat (version 3.10) and Prism^{R} (version 5.01). The One-way analysis of variance (ANOVA), followed by Student-Newman-Keuls tests were used to compare data, where value of $P < .05$ was considered statistically significant.

3. RESULTS

3.1 Determination of Sub-therapeutic Doses of *V. amygdalina*, Amodiaquine and Artesunate on Parasite Density.

The antimalarial effect of different doses of *V. amygdalina* (VA), amodiaquine (AQ) and artesunate (AS) on parasite density on days 4 and 7 post-treatment is presented in Tables 1, 2 and 3, respectively. Dose dependent chemosuppression was reveled for each of the treatments. The combination of AQ and AS resulted in 100% Chemo-Suppression effect (CSE) on day 7. The sub-therapeutic doses of VA, AQ and AS were found to be 100 mg/kg, 2 mg/kg and 2.4 mg/kg, respectively. These sub-therapeutic doses were used to assess the chemotherapeutic interaction of *V. amygdalina* with the conventional antimalarial drugs in subsequent combination treatments.

TABLE 1: Antimalarial activities of extract of *V. amygdalina* (VA) leaf extract at different doses in an early malarial infection in mice

Dose of *V. amygdalina* (mg/kg/day)	Average % CSE ± SEM (Day 4)	Average % CSE ± SEM (Day 7)
100	34.14 ± 6.6 ^{a,b,c,d}	51.84 ± 5.0 ^{a,b,c}

200	39.25 ± 8.2 [e]	61.49 ± 5.7 [d]
300	47.57 ± 6.2 [a]	64.95 ± 4.7 [a]
400	51.11 ± 7.6 [b]	68.63 ± 5.0 [b]
500	53.96 ± 6.8 [c,e]	70.87 ± 5.7 [c]
Normal saline	0.00	0.00

Values with the same superscript letters on days 4 and 7 respectively are significantly different (P < .05) while values with different superscript letters are not significantly different (P > .05)

TABLE 2: Antimalarial activities of Amodiaquine (AQ) at different doses in an early malarial infection in mice

Dose of Amodiaquine (mg/kg/day)	Average % CSE ± SEM (Day 4)	Average % CSE ± SEM (Day 7)
2	42.12 ± 5.4[a,b,c,d]	78.05 ± 2.0 [a,b,c,]
4	66.75 ± 3.4[d]	83.71± 2.1[d,f]
6	69.95 ± 5.0[c]	89.86 ± 2.1[a,c,]
8	72.15 ± 5.0[b]	99.01± 0.3[c,d]
10	74.42 ± 3.4[a]	100 ± 0.0 [a,f]
Normal saline	0.00	0.00

Values with the same superscript letters on days 4 and 7 respectively are significantly different (P < .05) while values with different superscript letters are not significantly different (P > .05)

TABLE 3: Antimalarial activities of Artesunate (AS) at different doses in an early malarial infection in mice

Dose of Artesunate (mg/kg/day)	Average % CSE ± SEM (Day 4)	Average % CSE ± SEM (Day 7)
0.8	18.89 ± 4.9 ^{a,b,c}	48.38 ± 2.0 ^{a,b,c}
1.6	22.92 ± 9.6 ^{d,e}	63.69 ± 3.3 ^{a,b,c}
2.4	53.32 ± 6.6 ^{a,b,d}	75.54 ± 2.8^{b,c}
3.2	80.47 ± 2.4^{a,d}	99.01 ± 0.4^{b}
4	86.32 ± 1.6^{b,e}	100 ± 0.0^{c}
Normal saline	0.00	0.00

Values with the same superscript letters on days 4 and 7 respectively are significantly different (P < .05) while values with different superscript letters are not significantly different (P > .05)

3.2 Effects of *V. amygdalina* and its Combination with Artesunate and/or Amodiaquine on parasite density in Early Infection

The antimalarial effects of sub-therapeutic doses of *V. amygdalina* (VA), amodiaquine (AQ) and artesunate (AS) when administered individually and in combinations on parasite density in early malaria infection are shown in Table 4. Significant (*P* = .01) parasite reductions were observed with concurrent administrations of the sub-therapeutic doses of VA (100 mg/kg) with AQ (2 mg/kg) or with AS (2.4 mg/kg) at day 4 post-infection in comparison with the effects of the extract or drug administered alone. For example, the chemosuppressive effect (CSE) of VA+AQ was 77.82% which is significantly (*P* =.01) higher than the values for the individual administrations of AQ (50.91%) or VA (36.15%). Similarly, the CSE of VA+AS (85.98%) was significantly (*P* =.01) higher compared to the effects of AS (61.64%) or VA alone. Also, significant differences ($p<0.05$) in the CSE values were demonstrated when determined on the day 7 post-infection but the magnitude of differences were not as much as was observed at day-4. Both combinations, VA+AQ+AS and AQ+AS gave 100% chemosuppression.

TABLE 4: Chemosuppressive Effect of *V. amygdalina* (VA) and its Combination with Amodiaquine (AQ) and/or Artesunate (AS) in an early malarial infection in mice

Dose of drugs (mg/kg/day)	Average % CSE ± SEM (Day-4)	Average % CSE ± SEM (Day 7)
VA(100)	36.15 ± 7.8 [a,b]	59.78 ± 5.4 [a,b]
AQ(2)	50.91± 6.1[a]	82.61 ± 2.8[a]
AS(2.4)	61.64 ± 4.3 [b]	87.21± 2.7[b]
VA+AQ	77.82 ± 1.7[a]	97.53 ± 0.5[a]
VA+AS	85.98 ± 1.4 [b]	98.62 ± 0.3[b]
VA+AQ+AS	100 ± 0.0	100 ± 0.0
AQ+AS	98.13 ± 0.5	100 ± 0.0
Normal saline	0.00	0.00

[a]VA+AQ Effect is significantly higher ($p < 0.05$) than that of AQ alone or VA alone

[b]VA+AS effect is significantly higher ($p < 0.05$) than that of AS alone or VA alone

3.3 Effect of *V. amygdalina* and its Combination with Artesunate and/or Amodiaquine on parasite density in Established Infection

The results of the antimalarial effects of the separate and concurrent administrations of *V. amygdalina* (VA), amodiaquine (AQ) and artesunate (AS) in established infection are presented in Table 5. Only the combination of VA+AQ showed a significant (P =.05) increase in the curative effects at the days 4 and 5 of treatment compared to the effects of treatment with VA or AQ alone. Also, VA enhanced the curative effect of AS at treatment days 4 and 5, but the enhancements were not significant.

Table 5: Curative Effect of *V. amygdalina* (VA) and its Combination with Amodiaquine (AQ) and/or Artesunate (AS) in Established Infection

Dose (mg/kg/day)	**Parasite Clearance (%) DAYS OF TREATMENT**			
	Day 2	**Day 3**	**Day 4**	**Day 5**
VA(100)	14.11 ± 4.6	28.57 ± 3.4	38.92 ± 3.7[a]	49.62 ± 3.3[b]

AQ(2)	29.55 ± 4.2	53.76 ± 2.7	67.73 ± 3.0[a]	78.44 ± 3.4[b]
AS(2.4)	34.04 ± 6.2	66.25 ± 2.1	76.64 ± 1.3	86.88 ± 4.1
VA+AQ	31.98±3.9	62.60 ± 5.4	79.26 ± 2.4[a]	90.03 ± 4.9[b]
VA+AS	30.23 ± 4.1	61.71 ± 4.1	79.83 ± 3.0	91.85 ± 1.3
VA+AQ+AS	60.47 ± 1.9	90.88 ± 1.4	98.54 ± 0.4	100 ± 0.0
AQ+AS	58.77 ± 5.4	88.73 ± 1.8	97.39 ± 0.5	100 ± 0.0
N. Saline	0.00	0.00	0.00	0.00

[a]*VA+AQ effect is significantly higher (p <0.05) than effect of VA or AQ alone at day-4 of treatment.*

[b]*VA+AQ is significantly higher (p <0.05) than effect of VA or AQ alone at day-5 of treatment.*

In the experiment to determine the mean survival period of the animals with established infection, VA alone or with its different combinations significantly prolonged the survival of animals when compared with normal saline (Table 6). Combination of VA with the conventional antimalarial drugs did not result in enhancement of the duration of survival compared to the effect of the synthetic drugs alone.

Figure 1 shows the survival percent observed in the various treatment groups. Expectedly, the treatment group of VA+AQ+AS had the highest survival rate but this was comparable to the effect of AQ+AS treatment.

Table 6 Mean survival time of *V. amygdalina* (VA) and its Combination with Amodiaquine (AQ) and/or Artesunate (AS) in Established Infection

Treatment (Dose) (mg/kg/day)	Mean ± SEM of Survival Time (days)
VA(100)	19 ± 1.3[b]
AQ(2)	23 ± 2.7[b]
AS(2.4)	21.8 ± 2.9[b]
VA+AQ	22.2 ± 2.5[b]
VA+AS	20.8 ± 2.4[b]
VA+AQ+AS	26.4 ± 1.6

AQ+AS	26.4 ± 1.6
N. Saline	13 ± 1.4[a]

Mean with different superscripts are significantly different from each other (P<0.05)

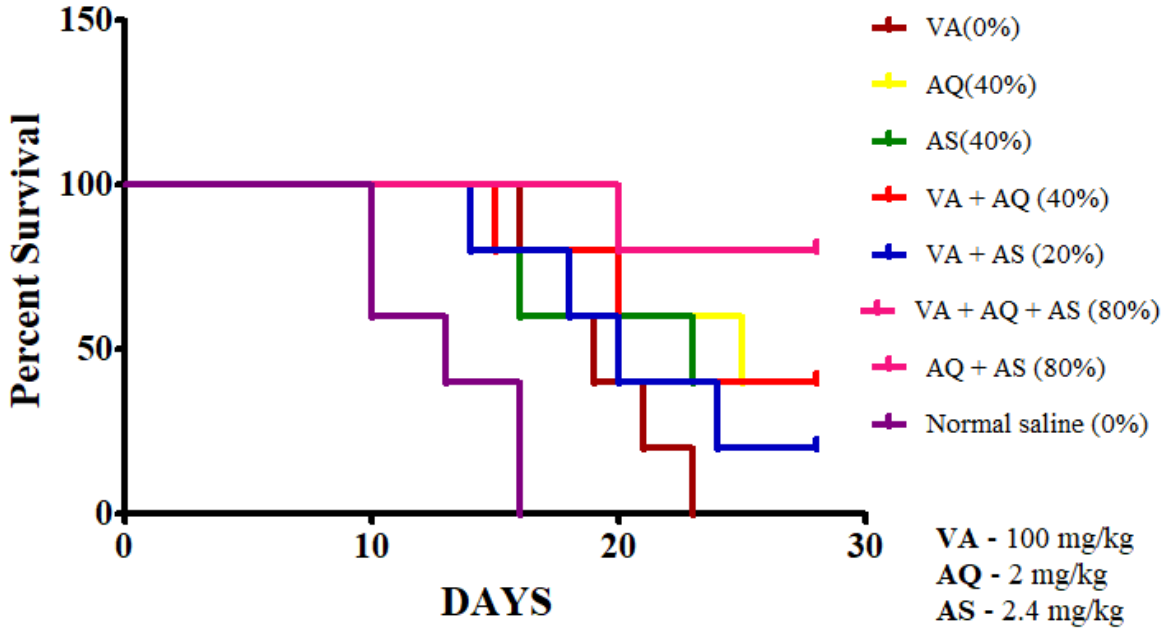

Fig. 1. Percentage Survival of Animals in Established Infection using the Kaplan-Meier Survival Curve

4. DISCUSSION

The incidence of concurrent use of orthodox antimalarial drugs with herbs is on the rise [11, 13]. Therefore, the need to assess and verify the nature of herb-drug interaction [18] that could be emanating from this practice has become essential. It was considered necessary to investigate the effect of a possible antimalarial chemotherapeutic interaction of *V. amygdalina* aqueous leaf extract because its antimalarial effects have been reported by several authors [8, 9, 10] including a clinical trial done by Challand and Willcox [11] which showed positive intervention in malaria chemotherapy. The clinical study done by Challand and Willcox [11] where freshly made infusion of *V. amygdalina* was administered daily for 7 days concluded that the herb seems to be safe and moderately clinically effective for malaria treatment.

The level of parasite infection in this study was expressed as the number of parasites per microlitre of blood (parasite density), rather than the usual percentage parasitaemia as this approach appears to have gained a wider clinical application [11, 19]. Parasite density expresses the level of infection and response to treatment and is defined as the measure of asexual parasites per microliter of blood (parasites/µl).

The highest dose per day of VA was set at 500 mg/kg based on studies by Abosi and Raseroka, and Challand and Willcox [9, 11]. In the present study, the inability of VA to achieve complete chemosuppression (Table 1) even at the highest dose may suggest that a longer duration of administration is required. That the malaria chemotherapeutic efficacy of VA extract depends on its duration of administration has also been suggested by Challand and Willcox [11] based on their observation of recrudescence in patients treated with VA for 7 Days. This incomplete parasite suppression by the aqueous extract of VA as observed in this study is also in agreement with the report of Abosi and Raseroka where the antimalarial activity of ethanol leaf extract of VA at different doses (125, 250, 500 mg/kg) showed a dose dependent effect without complete parasite suppression/clearance [9].

The Chemosuppressive effects (CSE) of the selected doses of AQ were dose dependent (Table 2) and this is consistent with the observations reported by Adepiti *et al*. in which AQ was used in combination with a herbal antimalarial product -MAMA decoction [20]. It is not surprising that AQ (10 mg/kg) and AS (4 mg/kg) exhibited 100% CSE on day 7 post-infection (Tables 2 and 3) since their antimalarial efficacies at these dosages are well documented [21]. In early malaria infection, the significantly higher CSE of AQ and AS when combined separately with VA suggests additive effect and this is in consonance with the results of a study which showed that the combination of VA and chloroquine resulted in a CSE that was higher than that of chloroquine alone [22].

In established malaria infection, the parasite clearance of AS when administered alone was not significantly ($P > 0.05$) different from that of its combination with VA on all treatment days confirming the rapid cure rate of AS in malaria infection [23]. On the other hand, the significantly increased parasite clearance effect of VA in combination with AQ also confirms the additive effect of VA when administered concurrently with AQ.

Although VA administration when compared to treatment with placebo (normal saline) significantly prolonged the duration of survival of the animals with established infection (Table 6), the complete absence of any survival after 28 days of monitoring suggests that the herbal extract should not be administered alone in malaria therapy especially for a short duration. This supports the suggestion by Challand and Willcox [11] of the need to study the antimalarial efficacy of VA extract for a longer duration. High survival rates of animals recorded in groups treated with VA+AQ+AS and AQ+AS, were not surprising as both combinations gave complete parasite clearance affirming the proven efficacy of the amodiaquine-artesunate combination [23]. The low survival percentage observed in other treatment groups may have emanated from the incomplete parasite clearance recorded, which may have led to recrudescence and death eventually. A similar observation of a low percentage survival of infected animals despite sustained clearance of parasitemia by amodiaquine has been previously reported [24]. As observed in the present study, despite the 100% parasite clearance observed in animals treated with VA+AQ+AS and AQ+AS, the survival rate was less than 100%. A lower percentage survival relative to a 100% parasite clearance can be attributable to the fact that the virulence factors already released or tissue/organ damages already caused by parasites can still result in the death of some of the infected animals even after complete clearance of the parasites [25, 26]. It is reported that sequestration of red blood cells infected with the plasmodium parasite within microvasculature of organs including the brain is an important mechanism for malaria pathogenicity in humans and rodent malaria models [27]. Therefore,

although the *P. berghei* rodent model is an adequate tool for research on malaria chemotherapy [27], studies in humans are still required to verify and confirm results from animal studies.

Overall, concurrent administrations of *V. amygdalina* with amodiaquine and/or Artesunate result in significant enhancement of the efficacies of these orthodox antimalarial drugs. This may be an approach towards overcoming development of resistance to malaria parasites by these drugs.

5. CONCLUSION

This study concluded that the co-administration of *V. amygdalina aqueous* leaf extract with either amodiaquine and/or artesunate is associated with significant enhancement of the antimalarial efficacy of the orthodox drugs. This study further demonstrates the potential benefits in combining conventional antimalarial drugs with herbal products. The reported efficacy of the amodiaquine-artesunate combination compared to the administration of the individual component drug alone was also underscored in this study. This investigation also buttresses the need for further exploration of antimalarial herb-drug co-administration.

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