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Evaluation of antiplasmodial potential of *Aloe barbadensis* and *Allium sativum* on *plasmodium berghei*-infected mice

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A significant percentage of the developing countries are living in malaria-endemic communities and are prone to contacting the disease. The fight against malaria is faced with the occurrence of widespread resistance of *Plasmodium falciparum* to the available anti-malarial drugs. Thus, this study evaluates the anti-malarial activity of extracts of *Aloe barbadensis* and *Allium sativum* in *Plasmodium berghei* infected mice. To achieve this, crude extracts of *A. sativum* and *A. barbadensis* were prepared. These extracts were administered orally to *P. berghei* infected mice after 24 h of infection at the concentration of 400, 600 and 800 mg/kg/day respectively for three days following the modified Peter four- day suppressive test procedure. The clearance rates in *P. berghei* infected mice were determined. Following the internationally accepted principles for laboratory animal use, the blood sample of the experimental mice was collected through cardiac puncture and diluted with normal saline and the experimental mice were infected intravenously. Parasitaemia infection was confirmed using 10% Giemsa-stained thin film fixed with methanol. Clearance rate was evaluated using the same staining procedure after three days of treatment. *A. barbadensis* extract showed a clearance rate A. barbadensis extract showed a clearance rates (% parasitaemia) of 6.3, 18.3 and 32.0% respectively at 400 mg/kg concentration, 5.3 to 28.7% for 600 mg/kg concentration and 4.0 to 22.3% at 800 mg/kg concentration. Also, with Allium sativum, the clearance rates increased from 8.3% to 26.1%at 400mg/kg, 7.7%to 25.1%at 600mg/kg and 6.9% to 24.3%at 800mg/kg respectively. Each extract shows variable level of parasitaemia suppression in dose related manner, when compared with the control groups.

Key words: Antiplasmodial potential, *Aloa barabadensis*, *Allium sativum*, *Plasmodium berghei*.

INTRODUCTION

The incidence of malaria is increasing, and there is an urgent need to identify new drug targets for both prophylaxis and chemotherapy (WHO, 2014). The epidemic is on the increase due to increase in resistance of the parasites to the available drugs as well as unavailability of vaccines against the spread of malaria in developing countries. The biggest challenge yet to the effective control of malaria is combating drug resistance. The parasite responsible for malaria (*P. falciparum*) is becoming resistant even to Artemisinin-based combination therapy (ACT) (Obidike et al., 2013). Malaria is one of the most important infectious diseases in the

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world. Each year 300 to 500 million new cases are diagnosed and approximately 1.5 million people die of the disease. More than 40% of the world's population live in malaria-endemic areas and are at risk of contracting the disease (WHO, 2016). The traditional medicine is estimated to be used by 80% of the population of most developing countries (Abeku, 2007) and these herbal medicines are used for primary health care needs. Although plants are unique in their activities, it has also been found that a particular plant may be used by different tribes or countries for treatment of different ailments. This shows that plants possess a very wide range of biological activities which are attributed to their chemical composition (Edeoga et al., 2005). There is increase resistance to commonly used drugs for malaria treatment in developing countries (Kumar et al., 2009). However, not much has been done to project anti-malarial properties of indigenous plants most especially in Africa (Abosi and Raseroka, 2003). This study was aimed at evaluating the anti-malarial potential of *A. barbadensis* and *A. sativum* as medicinal plants and if found effective, may be useful for the production of tolerable, less toxic and cheap anti-malarial drugs.

MATERIALS AND METHODS

This study was carried out in the animal house, Ladoke Akintola University of Technology College of Health Science, Osogbo.

Sample collection and processing

The research was conducted in accordance with the internationally accepted principles for laboratory animal use and as found in the U.S. guidelines. The mice were purchased from Animal House Ladoke Akintola University of Technology (18g-22g body weight) and *P. berghei* was provided by INMRAT (Institute Of Nigeria Medical Research Advance Technology) in University College Hospital, Ibadan. The mice were infected with *P. berghei* in the same research institute.

The parasite and infection

For *in vivo* anti-malarial assays, chloroquine (CQ) resistances ANKA strain of *P. berghei* was used. The parasite was maintained by serial passage of blood from infected mice to non-infected ones on weekly basis. To infect the mice, blood sample was collected through cardiac puncture of an experimental mouse with a rising parasitaemia of about 24%. Then, the blood was diluted in normal saline; so that each mouse was passaged with 0.2 ml of the infected blood containing about$1x10^7$ *P. berghei* parasitized red blood cells via intraperitoneal route (IP). The mice were housed in plastic cages in the animal house, Pharmacology Laboratory LAUTECH, Osogbo. They were kept under standard twelve hours light and twelve hours dark schedule where room temperature, humidity and ventilation were controlled during the acclimatization period of seven days. The mice were randomly placed into five groups of seven mice each for each treatment including control groups (positive control and negative control), which made up of seven mice in each group. The positive control group was given 0.2 ml of 10 mg/kg/mouse chloroquine (CQ) as a standard drug control, while the negative control group was given 0.5 ml distilled water per mouse. The test extracts were prepared in three different doses (400, 600 and 800 mg/kg of body weight). They were administered daily via the oral route to mimic its use in the folklore. The mice were fed with commercially purchased pallet food. Water was given *ad libitum* while food was withdrawn eight hour prior to treatment to ensure effective absorption from the gastrointestinal tract after oral administration (Coppi et al., 2006). Food was re-introduced thirty minute after treatment and withheld for a further 3-4 h after administration of the extracts as described by Peter et al. (1976). Each extract was administered as a single dose per day for three days. All the treatments were administered orally by using oral cannula. Treatment was started after 24 h of infection on day 0 and was continued daily for three days (from day 0 to day 3). On the seventh day blood sample was collected from the tail of each mouse. Thin smears were prepared and stained with 10% Geimsa stain solution. This was repeated on days 10 and 14, respectively. Then each stained slide was examined under the microscope with an oil immersion objective of 100x magnification power to evaluate the percent suppression of each extract with respect to the control groups. The parasitaemia level was determined by counting the number of parasitized erythrocytes out of 200 erythrocytes in random fields of the microscope. Average percentage chemo-suppression was calculated using this formula:

Average % suppression = 100- average parasitemia in test group x 100 average parasitemia in negative control

Collection of plant materials and extract preparation

Clean garlic bulbs specimens were collected from Oja-Oba Market, Ibadan and sent to the Botany Department of Obafemi Awolowo University of Ile-Ife for identification. The cloves of *Allium sativum* were thoroughly ground and macerated using blender with warm water at 45°C for 15 min and were stirred continuously to form a liquidized material. It was sieved to remove the shaft. 100 ml of water was added to 100 g of cloves according to Koshimizu et al. (1994). The liquid extract was stored at –4°C pending the time it will be used. *Aloevera* leaves were purchased in the Botanical Garden of Obafemi Awolowo University Ile-Ife and identification was done in the same institution. 25 g of *Aloevera* leaves was collected from the base of the plant. The leaf's skin was peeled off with the use of knife. The translucent gel was scooped out and blended in a blender. The gels were blended and poured into clean, sterile Petri dishes and put in a hot air oven for 18 h at 40°C for evaporation of the fluid until it became dry. The powder was scraped out of the Petri dishes and blended again to get a desired texture which was kept in a safe, dry container that has a cap. The powder was afterwards reconstituted with distilled water making it ready for proper use. Healthy physically alright mice, weighing between 18 to 22 g were used. Unhealthy, physically deranged and those that weighed below 18 g and above 22 g were not used.

RESULTS

Results of the study were expressed as mean±standard error of mean (M±SEM). Comparison of parasitaemia between tests and control groups and their statistical significance were determined by student t-test. All data were analyzed at a 95% confidence interval (P=0.05). Percentage parasitaemia and percentage suppression were also calculated. On day one, the mice were infected intravenously with diluted blood containing *P. berghei* as

Table 1. Parasitaemia level within 24 h of infection.

Group	A	B	C	D	E	F	I	J
	%							
Mouse 1	4.5	11	15	9.5	15	8.5	12.5	7.5
Mouse 2	12.5	12.5	8	8.5	7.5	10.5	10	10
Mouse 3	12.5	11.5	8.5	16	9.5	13.5	9	13.5
Mouse 4	13.5	13.5	11.5	8	11	11.5	13	12.5
Mouse 5	15	7.5	12.5	10.5	13.5	8	8.5	9
Mouse 6	15	13.5	13	13.5	10	10.5	7.5	13
Weight (g)	20.4	19.1	20.3	19.3	20.6	20.4	18.8	18.5

Table 2. Distribution of test and control groups of experimental mice at different concentration of the extracts.

Test groups	Concentration (mg/kg)	% Parasitaemia (days)			SEM (days)			P value
		7	10	14	7	10	14	
Aloe vera test	400	6.3	18.3	32.0	6.3±0.73	18.3±1.87	32.0±2	<0.05
	600	5.3	17.4	28.7	5.3±0.63	17.4±1.23	28.7±1.5	<0.05
	80	4.0	12.5	22.3	4.0±1.07	12.5±0.89	22.3±1.3	<0.05
Garlic test groups	400	8.3	19.9	26.1	8.3±0.77	19.9±1.90	26.1±1.5	<0.05
	600	7.7	18.3	25.1	7.7±0.59	18.3±1.56	25.1±1.2	<0.05
	800	6.9	10.4	24.3	6.9±0.57	10.4±1.03	24.3±0.5	<0.05
Control groups	CQ 1	2.3	10.4	21.3	2.3±0.88	10.4±1.46	21.3±1.1	-
	D/W	33.8	36.1	45.2	33.8±1.77	36.1±3.31	45.2±1.7	-

CQ, chloroquine (positive control); D/W, distilled water (negative control).

Parasitaemia level was established in each mouse after 24 h as shown in Table 1. There was no mortality observed in the mice even after 24 h of infection. Table 3 shows the relationship between the different doses or concentration of *A. barbadensis* and *A. sativum* extracts, negative and positive controls in relation to the level of parasitaemia inhibition of the extract after three days of treatment with *A. barbadensis* and *Allium sativum* extracts. Table 1 shows the effect of *A. barbadensis* and *A. sativum* extracts on established malaria infection. The result of *in vivo* evaluation of the *A. barbadensis* and *A. sativum* extracts on established infection showed a reduced parasitaemia level when compared with the negative control. For groups treated with *A. barbadensis* extract, mean parasitaemia with *P. berghei* infected mice ranged from 4±1.07 to 6.3±0.73 on day 7, whereas the corresponding figure in the control groups; positive control treated with chloroquine was 2.3±0.88 and negative control treated with distilled water was 33.8±1.77 on day 7. The extract induced statistically significant inhibition of parasitaemia in all the doses tested compared to the negative control ($P<0.05$). For groups treated with *A. sativum* extract, mean parasitaemia with *P. berghei* infected mice ranged from 26.1±1.5 to 8.3±0.77 on day 14. The extracts induced statistically significant inhibition of parasitaemia in all the doses tested compared to the negative control ($P<0.05$). Values were presented as Mean±SEM, n=6. Days 10 and 14 results are also given in Tables 2 and 3, respectively. Also, the result of *in vivo* evaluation of the *A. barbadensis* and *A. sativum* extracts on established malaria infection showed a dose-dependent clearance or chemo-suppressive activity (Table 3). With the treatment of *A. barbadensis* extract at 400 mg/kg the suppression effect was 81%, at 600 mg/kg it was 84%, at 800 mg/kg it was 88% and the positive control CQ at 10 mg/kg it was 93% on day 7. Then, treatment with *A. sativum* at 400 mg/kg exerted 75%, at 600 mg/kg exerted 77%, at 800 mg/kg exerted 80% and positive control CQ at 10 mg/kg exerted 93% parasite clearance or suppression after seven days of treatment with *A. sativum* extract as shown in Table 3. There was reduction in the clearance rate of the parasite with the extracts and the standard drug used (CQ at 10 mg/kg). Also, after day 7, moderate suppressive effect was

Table 3. Effect of *Aloe barbadensis* and *Allium sativum* extracts on established malaria infection.

Test groups	Concentration (mg/kg)	% Suppression (days)		
		D7	D10	D14
Aloe vera test groups	400	81	49	30
	600	84	51	38
	800	88	65	52
Garlic test groups	400	75	41	43
	600	77	49	45
	800	80	71	47
Control groups	CQ 10	93	71	54
	D/W	0.5	0.5	0.5

observed both in the test and control groups.

DISCUSSION

In this study the extracts of *A. barbadensis* and *A. sativum* showed considerable anti-plasmodial properties. The anti-malaria activities of the extracts increased with increase in the concentration of the extracts. This is similar to the study carried out by Abu et al. (2014) and the study carried out on garlic (Coppi et al., 2006).

The parasitaemia suppressive effect of *A. barbadensis* can be hypothetically related with the presence of high concentration of anthraquinones and other quinoid compounds that are the characteristic constituents of the genus and in this species. The result of the present study shows that *A. barbadensis* probably has some intrinsic antimalarial activity from the percentage parasitaemia inhibition and suppression or clearance when compared to that of chloroquine which is the standard drug. This study also established a preliminary approach, the rationale for traditional use of this plant in Nigeria as a remedy for malaria infection.

The parasitaemia suppressive effect of *A. sativum* can be said to be due to the presence of high concentration of allicin (Edeoga et al., 2005) and thiosulfinates substance (Odugbemi et al., 2007) as well as other compounds that are the characteristic constituents of the genus *Allium* (Coppi et al., 2006).

The result of the present study (Table 3 and figure 4) showed that *A. sativum* has some intrinsic antimalarial activities by it percentage parasitaemia inhibition and suppression or clearance when compared to that of chloroquine which is the standard drug. It was observed that an average of one mouse died per test groups. In negative control group all the mice died before the end of the experiment because they were only given distilled water but none died in positive control group during the course of the experiment. Those that died in test groups were majorly those of lesser body weights. Possible causes may be as a result of competition for food, water and intrinsic individual resistance or susceptibility to the plasmodium. Also it was observed in those that died that they were feeling feverish, lacked appetite for food and water. They were sluggish in movement when excited. All these can be as a result of coming down with parasitaemia in those animals where probably extract utilization was slow.

The present study indicates that the extract possesses considerable in vitro antiplasmodial activity against plasmodium berghei infection relatively at high dose where low parasitemia was recorded.

In conclusion, This observation compares well with the advantages of polyherbal therapies over monotherapy (Madara et al., 2010). Similarly, (Odugbemi et al., 2007) reported that the two plants have been traditionally claimed to relieve fever and cure malaria. Crude extracts of *A. barbadensis* and *A. sativum* antimalaria activities against *P. berghei* infection probably indicated that these plants contain some antiplasmodial compounds (Sofowora et al., 2013). However, the crude extract should be further fractionated and tested for their activities against *P. falciparum* and *P. vivax* in order to consider them as potential sources for antimalarial agent for the treatment of human malaria. The fact that can be deduced from this study is that *A. barbadensis* and *A. sativum* possess antiplasmodium activities against *P. berghei.* It would therefore be worthwhile to purify the active compounds in these plants by fractionation and any other method of isolation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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