ORIGINAL ARTICLE

Discovery Phytomedicine 2019, Volume 7, Number 3: 112-117

Assessment of antiplasmodial activity of *Anthocleista grandiflora* on *Plasmodium berghei* infected mice

ABSTRACT

Medical plants are used traditionally as alternative treatment for malaria. *Anthocleista grandiflora* (*A. grandiflora*) is used traditionally for the treatment of malaria. This study attempt to scientifically validate its traditional use for malaria treatment by evaluating the antiplasmodial activity of its aqueous leaf extract (AAG) in *Plasmodium berghei* infected mice. *P. berghei* infected mice were orally treated with AAG (100, 200 and 400 mg/kg) daily in curative, suppressive and prophylactic studies. The untreated parasitized control (UPC) and the (PC) positive control were orally treated with normal saline (0.2mL) and chloroquine (CQ) (10mg/kg) respectively. At the end of treatment, blood samples were analyzed for parasitamia levels, hematological parameter and lipid profile. No morality was observed in acute toxicity evaluation of AAG in mice. AAG showed significant dose-dependent curative, suppressive and prophylactic activities via reductions in parasitamia levels at 100 mg/kg ($p<0.05$), 200 mg/kg ($p<0.01$) and 400 mg/kg ($p<0.001$) when compared to UPC. Mean survival time was increased in a dose-dependent fashion at 100 mg/kg ($p<0.05$), 200 mg/kg ($p<0.01$) and 400 mg/kg ($p<0.001$) when compared to UPC. AAG increased red blood cells, hemoglobin, pack cell volume, high density lipoprotein cholesterol levels, but decreased white blood cells, total cholesterol, triglyceride and low density lipoprotein cholesterol levels in a dose-dependent fashion at100 mg/kg ($p<0.05$), 200 mg/kg ($p<0.01$) and 400 mg/kg ($p<0.001$) when compared to UPC. Comparatively the effects of AAG (400 mg/kg) were statistically at par with (CQ) 10mg/kg. Based on the observations in this study, AGA seems safe and has antimalaria activity.

Keywords: *Anthocleista grandiflora,* Antimalaria, Hematology, Lipids, Mice

INTRODUCTION

Malaria is caused by mosquitoes which are host to protozoan parasites of the genus Plasmodium. Malaria is one of the primary infectious diseases of public health concern. It is a major health predicament especially in tropical and subtropical regions. Annually, over 500-550 million people suffer from malaria, leading to 1-2 million mortality with 90% mortality in children under the age of five in sub-Saharan Africa (Tangpukdee et al., 2009). Despite efforts to curtail malaria infection, increase numbers of cases are reported annually with most in sub-Saharan Africa which have been attributed to a number of factors (Pasvol, 2015). These factors include limited access to treatment, resistance to anti-malarial drugs and insecticides, residual and outdoors transmission and hard to reach population (Guyant et al., 2015). These factors have impacted negatively on the fight against malaria necessitating the quest for alternative malaria treatment including the use of plants (Muregi et al., 2003). Research for alternative anti-malarial drugs especially of plant origin has increased over the last two decades (Petros, 2011)

Plants have been used traditionally to treat malaria and are the primary sources of quinine and artemisinin derivatives which are the back bone of modern antimalarial drugs. Most people, particularly of African descent use plants traditionally to fight diseases. The current use of plants as remedies for ailments reflects attachment to culture and a lack of access to modern medicine (Randrianarivelojosia et al., 2003). Researches on medicinal plants have been the focus of discovering alternative anti-malarial drugs (Ntie-Kang et al., 2014). *Anthocleista grandiflora* (*A. grandiflora*), also known as forest fever tree, is a member of the family Gentianaceae. It is a tall and slender tree that is up to 30 m, which grows in forests with heavy rainfall, in patches of relict forest on hillside, swampy places, beside densely wooded streams in tropical and subtropical areas in East and South Africa. It also grows in Zanzibar and Comore Islands. It has very large leaves which are up to 100 cm × 50 cm, arranged in clusters (Schmidt et al., 2002; Notten, 2014). *A. grandiflora* is a multipurpose plant used traditionally as remedy for some ailments. The leaves and bark are use as malaria remedy whereas the bark is use as remedy for diarrhea, hypertension, diabetes, and venereal disease (Foden and Potter, 2005). The bark is also used to treat epilepsy, fever, and hepatitis. In vitro tests showed it has activity against pathogens that are of public health concern (Boon, 2010). Investigation of its stem bark showed the presence of phytochemicals of medicinal

significance (Magadulaa et al., 2008; Odeghe et al., 2012). This study attempt to scientifically validate the traditional use of its leaf as malaria remedy by evaluating the antiplasmodial activity of its aqueous leaf extract in *Plasmodium berghei* infected mice.

MATERIALS AND METHODS

Collection of plant material

The fresh leaves of *A. grandiflora* were collected during the dry season at Obia/Akpor Local Government Area of Rivers State, Nigeria. The leaves were identified with voucher number Intercedd 19111 at the International Centre for Ethnomedicine and Drug Development in Nsukka, Enugu State, Nigeria by Mr.Ozioko O a taxonomist. Chloroquine (10 mg/kg) (Sonsak *et al.,* 2018) used as standard control was manufactured by Evans Medical Plc, Nigeria

Preparation of plant material

The fresh leaves of *A. grandiflora* were washed and allowed to dry at room temperature. The dry leaves were powdered with a milling machine and 45g was soaked in water of 1.5 litres for 48 hours with intermittent shaking. The water extract was filtered using Whatman filter paper and heated over a water bath at 50°C to obtain an extract free of solvent.

Extract yield

A. grandiflora (300g) produced 27.2g of aqueous extract which represents an extract yield of 9.1%

Experimental animals

Swiss albino mice weighing 20-30g (8 weeks old) of both sexes were used. The mice were supplied by the animal breeding unit of the University of Nigeria, Nsukka, Enugu State, Nigeria. The mice were kept in cages (5/group) with free access to water and diet. The mice were kept at a temperature of 28.0 + 2.0oC and a 12 hour light/dark cycle. The mice were acclimatized for 2 weeks prior to the experiment.

Phytochemical analyses

The presence of tannins, terpenoids, carbohydrates, flavonoids, proteins, saponins, cardiac glycosides and alkaloids were determined using standard procedure (Harbone, 1973; Trease G and Evans, 2008)

Acute toxicity test

Acute toxicity study was performed in two phases 'phase 1 and phase 2' using modified Lorke's method (1983). In phase 1, 9 mice randomized into 3 groups of n=5 were used. The mice were orally treated with AAG (10, 100 and 1000 mg/kg) and examined for behavioral changes and mortality. In the absence of mortality in phase 1, phase 2 study was performed. In phase 2, 3 mice grouped into 3 of n=5 were used. The mice were orally administered with AAG (1500, 2000 and 2500 mg/kg) and were observe for behavioral changes and mortality.

Malaria parasite and animal inoculation

The chloroquine sensitive strain of *Plasmodium berghei* (*P. berghei*) (NK 65) used was obtained from from the Department of Pharmacology, Faculty of Basic Medical Sciences, University of Port Harcourt, Rivers State, Nigeria. Parasitized erythrocyte from a donor mouse was collected in EDTA-treated bottles. The blood samples (parasitized erythrocyte) were diluted with normal saline (0.2 mL). The experimental mice were inoculated intraperitoneally with the blood diluted blood sample containing 1×10^7 parasite load.

Antimalaral assessment of *A. grandiflora* extract

Curative study

Curatie study was performed as reported by Ryley and Peter (1970). Twenty five mice grouped into 5 of n=5 were used. The mice were inoculated with *P. berghei* (1×10^7) and parasitamia was confirmed after 72hours. The parasitized mice were treated with AAG (100, 200 and 400 mg/kg) once daily for 3 days. The untreated parasitized control (UPC) and the positive control (PC) were treated with normal saline (0.2mL) and CQ (10 mg/kg) once daily for 3 days respectively. On the 5th day (day 4), thin films were made from the tail blood of each mouse and smear on to a microscope slide to make a film. The blood films were fixed with methanol, stained with 10% Giemsa at pH 7.2 for 10 min and parasitemia examined microscopically. The parasitemia level was determined by counting the number of parasitized erythrocytes out of 200 erythrocytes in random fields of the microscope. Average percentage suppression was calculated as:

$$\%\ \text{Parasitamia} = \frac{\%\ \text{Parasitamia of untreated control} - \%\ \text{Parasitamia of treated group}}{\%\ \text{Parasitamia of untreated control}} \times 100$$

Suppressive study

Suppressive study was performed as reported by of Knight and Peters (1980). Twenty five mice grouped into 5 of n=5 were used. The mice were pretreated with AAG (100, 200 and 400 mg/kg) once daily whereas the UPC was treated with normal saline (0.2mL) and the PC was treated with CQ (10 mg/kg) for 4 days prior to inoculation with *P. berghei* (1×10^7). Treatment continues for 7 days, on day 8

blood samples were collected via the tail and evaluated for parasitamia as mentioned above

Prophylactic study

Prophylactic antip;asmodail effect of AAG was evaluated according to Peters (1967). Twenty mice randomized into 5 groups of n=5 were used. The mice were orally pretreated with AGA (100, 200 and 400 mg/kg) once daily for 4 days. On the hand, UPC was orally treated with normal saline (0.2mL) whereas PC was orally treated with CQ (10mg/kg) once daily for 4 days. Thereafter, the mice were inoculated with 1×10^7 *P. berghei* i.p and treatment continued for 8 days. On day 8 (day7), parasitemia was determined as described above.

Determination of mean survival time

Mean survival time (MST) was evaluated by daily monitoring of mice for mortality and the number of days from infection to death for the mice were recorded. MST was calculated using the formula below.

$$\text{MST} = \frac{\text{Sum of survival time of all mice in a group}}{\text{Total number of mice in that group}}$$

Evaluation of serum biochemical parameters

Total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), packed cell volume (PCV), hemoglobin (HB), white blood cells (WBC) and red blood cell (RBC) levels were evaluated with the aid of auto analyzer.

Acute toxicity test

Table 1 Phase 1 of acute toxicity test

Dose mg/kg	No of mice per group	No of death
10	3	0/3
100	3	0/3
1000	3	0/3

Table 2 Phase 2 of acute toxicity test

Dose mg/kg	No of mice per group	No of death
1500	1	0/1
2000	1	0/1
3000	1	0/1

RESULTS

The presence of more of tannins, terpenoids, carbohydrates, proteins, saponins, flavoniods alkaloids and less of saponins and cardiac glycosides were observed in phytochemically screened AAG. In this study, no behavioral changes and morality were observed in the acute toxicity study of AAG (Tables 1 and 2). The suppressive, curative and the prophylactic evaluations of EEA showed dose-dependent decreases in percentage parasitamia. Significant decreases in percentage parasitamia levels were observed at 100 mg/kg ($p<0.05$), 200 mg/kg ($p<0.05$) and 400 mg/kg ($p<0.001$) of AAG. CQ treatment also produced significant reductions in percentage parasitamia levels at $p<0.001$ when compare to UPC (Tables 2-5). In suppressive, curative and prophylactic evaluations of AAG, MST was increased in a dose-dependent fashion. The observed increases in MST were significant at 100 mg/kg ($p<0.05$), 200 mg/kg ($p<0.05$) and 400 mg/kg ($p<0.001$) of AAG when compared UPC. CQ treatment also increased MST significantly at $p<0.001$ when compared to UPC (Tables 2-5). RBC, Hb, PCV levels were increased whereas WBC levels were decreased in a dose-dependent fashion in mice treated with AAG with observed significance at 100 mg/kg ($p<0.05$), 200 mg/kg ($p<0.05$) and 400 mg/kg ($p<0.001$) when compared to control. Also, treatment with CQ produced significant increases in RBC, Hb, PCV levels with decrease in WBC level at $p<0.001$ when compared to UPC (Tables 6). Further, reductions in TG, TC and LDL-C with increases in DHL levels in a dose-dependent fashion occurred in parasitized mice treated with 100 mg/kg ($p<0.05$), 200 mg/kg ($p<0.05$) and 400 mg/kg ($p<0.001$) of AAG when compared to UPC (Table 6).

DISCUSSION

Malaria is a primary and severe public health problem in the world. It is one of the leading causes of death in many developing countries, especially among pregnant women and young children. The development of drug-resistant strains of Plasmodium parasite, occurrence of adverse effects and the cost of existing anti-malarial drugs have instituted the experimental search for novel, efficient well tolerated antimalarial drugs from alternative sources including plants (Iyamah and Idu, 2015). Plants are a major component of traditional systems of medicine in developing countries, which are essential and integral aspect of their cultural practices and history. Medicinal plants are good sources of alternative medicines with tremendous potential and opportunities. Some plants especially herbs are medicinal and are used in rural settings where health care in not easily accessible for the prevention and treatment

Table 3 **Curative antimalarial activity of *A. grandiflora***

Treatment	% Parasitaemia ± SEM	% Inhibition	MST
(UPC) NS 0.2mL	43.7 ± 2.33	0.00	9.60
AAG 100mg/kg	26.8± 2.44 π	39.0	16.2 π
AAG 200mg/kg	24.4 ± 2.54*	58.7	26.7*
AAG 400mg/kg	18.0± 0.92**	75.2	38.4**
(PC) CQ10 mg/kg	2.80 ± 0.03**	93.6	43.3**

UPC; Untreated parasitized control, NS: Normal saline, AAG: Aqueous extract of ***A. grandiflora***, PC; Positive control; CQ: Chloroquine, MST: Mean survival time. π p<0.05 difference when compared to UPC, *p<0.01 when compared to UPC, **p<0.001 difference when compare to UPC

Table 4 **Suppressive antimalarial activity of *A. grandiflora***

Treatment	% Parasitaemia ± SEM	% Inhibition	M ST
(UPC) NS 0.2mL	43.7± 2.32	0.00	9.30
AAG 100mg/kg	26.7 ± 1.31π	38.8	15.2 π
AAG 200mg/kg	21.7 ± 2.44*	50.3	20.0*
AAG 400mg/kg	12.5± 2.74**	71.4	27.1**
(PC) CQ 10 mg/kg	4.55 ± 1.11**	89.6	40.6**

UPC; Untreated parasitized control, NS: Normal saline, AAG: Aqueous extract of ***A. grandiflora***, PC; Positive control; CQ: Chloroquine, MST: Mean survival time. π p<0.05 difference when compared to UPC, *p<0.01 when compared to UPC, **p<0.001 difference when compare to UPC

Table 5 **Prophylactic antimalarial activity of *A. A. grandiflora***

Treatment	% Parasitaemia ± SEM	% Inhibition	M ST
(UPC) NS 0.2mL	58.2± 3.90	0.00	9.30
AAG 100mg/kg	37.8 ± 3.72 π	35.1	11.3 π
AAG 200mg/kg	29.0 ± 2.22*	50.1	20.4*
AAG 400mg/kg	17.6± 1.65**	69.7	27.5**
(PC) CQ 10 mg/kg	8.7± 0.21**	85.1	39.7**

UPC; Untreated parasitized control, NS: Normal saline, AAG: Aqueous extract of ***A. grandiflora***, PC; Positive control; CQ: Chloroquine, MST: Mean survival time. π p<0.05 difference when compared to UPC, *p<0.01 when compared to UPC, **p<0.001 difference when compare to UPC

of several human diseases (Iyamah and Idu, 2015). *A. grandiflora* is a medicinal plant which leaves; root, stems, flowers and bark are used for the treatment of different ailments (Palmer and Pitman; 1972). In Southern Africa, the leaves are used traditionally to treat malaria (Palmer and Pitman; 1972) with no supportive scientific evidence. In an attempt to established scientific evidence for the traditional use of *A grandifolia* as an antimalaria, this study evaluated the antiplasmodial activity of its aqueous leaf extract (AAG) in *P. berghei* infected mice. Plants contain phytochemicals which are imperative for their nutritive and medicinal values. Phytochemical screening is one of the mirrors by which plant phytochemicals are seen and indentified. In this study, phytochemically screened AAG revealed the presence of more of tannins, terpenoids, carbohydrates, proteins, flavoniods, alkaloids and less of cardiac glycosides and saponins. This finding supports the possible medicinal value of AAG. In this study, acute toxicity assessment of AAG shows it may be safe due to lack of behavioral changes and mortality. Suppressive, prophylactic and curative test are used to assess the antiplasmodial activities of candidate drugs in animals (Olanlokun et al., 2017). The suppressive, prophylactic and curative antiplasmodial evaluations of AAG showed potential antimalarial activity due to decreases in percentage parasitamia in a dose-dependent fashion. Malaria associated mortality is a serious public health concern that is prevalent in endemic regions especially Africa. In 2018, statistically, South Saharan African accounted for 94% of malaria associated death in the world of which 67% of death occurred in children under the age of five (Ouédraogo et al., 2020). One of the primary objectives of antimalaria therapy in infected persons is the achievement of zero mortality. MST (Mean survival time) is an experimentally used tool for the assessment of the effectiveness of

Table 6 **Effect of *A. grandiflora* on hematological and lipid profile of parasitized mice**

Treatment	RBC	WBC	PCV	Hb	TC mg/dL	TG mg/dL	LDL mg/dL	HDL mg/dL
(NPC) NS 0.2mL	5.4	6.96	55.9	17.2	154.6	98.9	2.12	58.3
(UPC) NS 0.2mL	2.37	17.1	20.7	5.04	464.8	380.7	5.63	20.0
AAG 100mg/kg	3.00 π	13.2 π	26.8 π	7.81 π	351.1 π	300.0 π	4.41 π	27.1 π
AAG 200mg/kg	3.91*	10.4*	33.1*	10.9*	244.2*	217.1*	3.20*	36.9*
AAG 400mg/kg	4.90**	7.00**	49.6**	15.8**	171.8**	123.6**	2.45**	47.8**
(PC) CQ 10 mg/kg	5.26**	6.76**	53.8**	16.7**	164.6**	107.2**	2.32**	53.7**

NPC; Non-parasitized control, UPC; Untreated parasitized control, NS: Normal saline, AAG: Aqueous extract of ***A. grandiflora***, PC; Positive control; CQ: Chloroquine, MST: Mean survival time. π p<0.05 difference when compared to UPC, *p<0.01 when compared to UPC, **p<0.001 difference when compare to UPC

candidate drugs to reduced mortality in plasmodium parasite infected animals (Olanlokun et al., 2017). In the current study, suppressive, prophylactic and curative studies showed that's treatment with AAG increased MST in a dose-dependent manner. Malaria is a major cause of anaemia in tropical areas. Studies have reported a prevalence of 60%-63% of malaria induced anemia with highest prevalence in children of 1-5 years (Sumbele et al.,. 2016). Malaria infection causes anemia via hematological insults causing haemolysis of infected and uninfected erythrocytes and bone marrow dyserythropoiesis which compromises rapid recovery from anaemia (White, 2018). The anemic effect of malaria correlates with decreases in RBC, HB, and PCV with increase in WBC observed in this study in *P. berghei* infected mice. However, in a dose-dependent fashion, treatment with AAG curtailed the anemic effect of *P. berghei* by increasing RBC, HB, and PCV with decreasing WBC. Furthermore, impaired lipid profile marked by elevated TC, TG, LDL-C and decreased HDL-C levels were conspicuous in *P. berghei* infected mice. However, treatment with AAG stabilized lipid profile by decreasing TC, TG, LDL-C and increasing HDL levels. The antipasmodial activity of AAG may be due to its phytochemical constituents. Some of its constituents such alkaloid, tannins, flavonoids have been reported to have antiplamodial activities (Wele *et al.,* 2018; Uzor, 2020). Conclusion: AAG seems safe and showed potential antiplasmodial which supports traditional use as malaria remedy.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENT

We kindly appreciate the handling of animals by Mr Eze I hukwumere of the Department of Pharmacology/Toxicology, Faculty of Pharmacy, Madonna, University, Nigeria. We also appreciate Mrs Matilda Deko of the Department of Pharmacology, Faculty of Basic Medical Sciences, University of Port Harcourt, Rivers State, Nigeria for the supply of Plasmodium parasite.

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