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Research Article

COMPARATIVE STUDY ON THE ANTIMALARIAL ACTIVITY OF METHANOL EXTRACT OF *Salacia senegalensis* LEAF LAM (DC) *VIS-À-VIS* CHLOROQUINE AND ARTESUNATE IN ALBINO MICE INFECTED WITH CHLOROQUINE-SENSITIVE *Plasmodium berghei* (NK65)

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Abstract

The comparative study on the antimalarial activity of methanol extract of *Salacia senegalensis* leaf were evaluated in albino mice infected with chloroquine-sensitive *Plasmodium berghei* (NK65) in order to justify its activity or performance as antimalarial remedy in Nigerian folk medicine *vis-à-vis* standard drugs(chloroquine and artesunate). Activities evaluated were suppressive effect, curative effect and prophylactic effect. Results of the antimalarial effect of methanol extract of *Salacia senegalensis* leaf evaluated in albino mice infected with chloroquine-sensitive *Plasmodium berghei* (NK65) showed a dose dependent blood schizontocidal activity at all the phases of malarial infection studied. The *in vivo* antimalarial effect of the extract (1000, 1200 and 1400 mg/kg body weight) against *P. berghei* showed significant ($p < 0.05$) dose-dependent activity for suppressive, curative and prophylactic test. When the extract dose increased from 1000 to 1400 mg/kg/day, chemosuppressive effect of the extract increased from 66.47 % to 80.33 %. There was also an increase from 66.57 % to 75.41 % and from 64.90 % to 82.72 % for the repository and curative activities respectively. The schizontocidal performance were comparable to that of chloroquine which had percentage suppression of parasitaemia as 87.03 %, 85.12 %, and 91.68 % and artesunate which had percentage suppression of parasitaemia as 88.39 %, 86.01 % and 93.83 % for suppressive, prophylactic and curative activities respectively. The percentage mean survival time of the *P. berghei* infected mice treated with extract doses were comparable to that treated with the standard drugs- chloroquine and artesunate. The result showed that the herbal extract possesses significant antimalarial potency which was comparable to that of standard antimalarial drugs used.

Keywords: Antimalarial, *Salacia senegalensis, Plasmodium berghei*, chemosuppression, chloroquine, artesunate

Introduction

The burden of malaria caused by *Plasmodia* is a world's health challenge. It has remains a major health burden to Africa and Nigeria, despite various declarations by African governments in the main context of the Roll back Malaria. In Nigeria, the burden of malaria is well documented and has been shown to be a huge contributor to the economic burden in communities where it is endemic and is responsible for annual economic loss of 13 billion Naira (WHO, 2009; Onwujekwe, *et al*., 2000). It is estimated that 300,000 deaths occur each year, and 60 % of outpatient visits and 30 % hospitalizations are all attributed to malaria (FMOH, 2009). About 50 % of the population has at least one episode of malaria annually resulting in high productivity losses (FMOH, 2009; WHO, 1995). The disease is particularly virulent among pregnant women and children under 5 years of age due to their lower immunity levels (WHO, 2000). The trend is rapidly increasing due to the current malarial resistance to first line of antimalarial drugs like chloroquine and artesunate (WHO, 2000). It is responsible for over 90 % of reported cases of tropical disease in Nigeria (Alaba, 2005; WHO, 2005). The efficacy or performance of these first lines of drugs against malaria parasite has been reported with variable success (Meleney, 1982; Basau and Haldar, 1994). The toxic effects of these chemicals on humans

(Butenkotter and Kaemmerer 1973; Murray *et al.*, 1992), the development of resistance to it by target parasites (Maingi *et al*., 1996), and the high cost of drugs (Chema and Ward, 1990) have paved way for herbal remedies as reasonable alternative. Many plants of Nigeria origin including *Salacia senegalensis* have been found with amazing antimalarial properties (Adumanya, *et al*., 2014a). It is therefore very necessary that such plants used by the local people as antimalarial be scientifically investigated to prove their ethnotherapeutic activity or performance *vis-à-vis* first line antimalarial drugs like chloroquine and artesunate. Chloroquine is the first line treatment for malaria especially uncomplicated malaria. It is an antimalarial drug discovered in 1934 by Hans Andersag and coworkers at the Bayer laboratories, who named it "Resochin"(Krafts *et al*., 2012). It was ignored for a decade because it was considered too toxic for human use. During World War II, United States government-sponsored clinical trials for antimalarial drug development showed unequivocally that chloroquine has a significant therapeutic value as an antimalarial drug. It was introduced into clinical practice in 1947 for the prophylactic treatment of malaria (Centers for Disease Control, n.d). Despite the growing problem of resistance to this drug, chloroquine remains the first-line treatment for uncomplicated malaria in much of Africa (Kamya *et al*., 2001; Yeshiwondim *et al.*, 2010). Its efficacy as an antimalarial has been reported (Mejia-Torres *et al*., 2013). Artesunate an antimalarial drug is a semisynthetic derivative of artemisinin whose water solubility facilitates intestinal absorption (Barradell and Fitton, 1995) and provides an advantage over artemisinin because it can be formulated as oral , rectal, intramuscular, and intravenous preparations(Awad, *et al*., 2003). Efficacy of artesunate against *Plasmodia* has been reported (Borrmann, *et al.*, 2002; Hamedi, *et al.*, 2004; Haroon, *et al.*, 2005). *Salacia senegalensis* Lam (DC) is an erect or climbing shrub with white or pale greenish cream petals and orange or yellow flowers. It is found in tropical forests. It belongs to the family Celestraceae. Traditionally, the extract of its leaf is used in malaria treatment, as a lotion for sick children and in the treatment of skin problems like eczema by the people of South-East zone of Nigeria (NNMDA, 2011). Recently its scientific antimalarial property was reported (Adumanya *et al.*, 2014a). But its comparative activity/performance with standard drugs like artesunate and chloroquine is yet to be reported. Therefore, the comparative study on the antimalarial activity/performance of methanol extract of *Salacia senegalensis* leaf were evaluated in albino mice infected with chloroquine-sensitive *Plasmodium berghei* (NK65) in order to justify its activity or performance as antimalarial remedy in Nigeria folk medicine *vis-à-vis* standard drugs (chloroquine and artesunate).

Materials and Methods

Plant Materials collection and authentication

The plant *Salacia senegalensis* (**figure 1**) was obtained from the forest at Orji, Owerri North L.G.A, Imo State, Nigeria, identified and authenticated by taxonomists Prof. Okeke, SE and Dr. Mbagwu, FN of the Department of Plant Science and Biotechnology Imo State University, Owerri, Nigeria.

Extraction procedures

Salacia senegalensis leaves were cleaned, cut into pieces and air dried at room temperature. Dry leaves were grounded into a coarse powder using a mortar, and milled to fine powder using electric blender (Q-link). Five hundred grams (500 g) of the powder was macerated in 1600 ml of 95 % methanol for 72 hours. The methanol extract was concentrated using rotary evaporator at temperature of 45-50 ^{0}C.

Mouse strain

Albino mice (healthy ones) of both sexes weighing between 18- 22 g were obtained from the Animal House of the Department of Biochemistry, University of Port Harcourt. The mice were appropriately grouped and kept in plastic cages and allowed to acclimatize for a period of one week before the commencement of the study. They were allowed unrestricted access to standard feed (Vital feed growers) obtained from Brand Cereals and Oil Mills Ltd, Bukuru, Jos, Nigeria and water *ad libitum* throughout the experimental period. The mice were handled in accordance with the guidelines for the care and use of laboratory animals (US- NRC, 2003).

Acquisition of Malaria Parasite

Already parasitized albino mice with chloroquine-sensitive *Plasmodium berghei (NK65)* were obtained from National Institute for Medical Research (NIRM), Lagos, Nigeria and maintained in the laboratory by serial passage in mice.

Inoculation with *P. berghei* Parasite

The parasitized albino mice were used as donors. Their parasitaemia levels were first determined and their blood diluted with normal saline. Then 0.2ml of the diluted blood (contained 1 x 10 ^{7} *P. berghei* infected red blood cells) was administered intra-peritoneally to each test mouse.

Safe dose and acute toxicity (LD_{50})

LD_{50} of *Salacia senegalensis* showed that a dose of less than or equal to 5000 mg per kg body weight (b.w) is safe, i.e. no death was recorded at this maximum concentrations used, but extremely high doses may not be advisable (Adumanya *et al*., 2014b). It showed that LD_{50} > 5000 mg/kg b.w.

***In vivo* test**

Salacia senegalensis methanol leaf extract and the standard drugs chloroquine phosphate tablets obtained from Dana Pharmaceuticals Nig. Plc. and artesunate obtained from Mekophar Pharm. Company Vietnam respectively were administered orally using sterile orogastric tubes.

Chemosuppressive Effect: Evaluation of schizontocidal activity on early infection

The Knight and Peters (1980) 4- day suppression test was used to determine the chemosuppressive effect as reported by Adumanya, *et al*., (2014a). Percentage parasitaemia was calculated using the formula:

$$PP = \left(\frac{\text{Total No. of PRBC}}{\text{No.of RBC}} \right) X100 \quad \text{.....(a)}$$

Where, PP = Percentage parasitaemia, PRBC = Parasitized red blood cells, RBC=Red blood cells

The percentage suppression of parasitaemia was then calculated with respect to the control group using the formula:

$$A = \left(\frac{B - C}{B} \right) X100 \quad \text{.....(b)}$$

Where, A = average percentage suppression of parasitaemia, B = average percentage parasitaemia in control group (normal saline), C = average percentage parasitaemia in Test group

Repository (Prophylactic) Effect: Evaluation of schizontocidal activity on residual infection

The method described by Peters (1967) was used to determine the repository activity of the extract. Then the average percentage suppression of parasitaemia was calculated using the formula (**b**) above.

Curative Effect: Evaluation of schizontocidal activity in established infection.

Modified method similar to that of Ryley and Peters (1970) was used to determine the curative activity of the extract as reported by Adumanya *et al*., (2014a). Average percentage suppression of parasitaemia was calculated using the formula:

$$A = \left(\frac{D - E}{D} \right) X\,100 \quad \text{.....(c)}$$

Where, A = average percentage suppression of parasitaemia, D = average percentage parasitaemia before treatment and
E= average percentage parasitaemia after treatment

Statistical Analysis

Results of the study were presented as means ± standard deviation. Statistical Package for Social Sciences (SPSS) version 20.0 was used in the statistical analyses and the means compared at 95 % level of confidence.

Results and Discussion

A dose-dependent effect (Table 1, 2, and 3) were observed on comparative activities of the chemosuppressive, prophylactic and curative tests respectively. At extract doses of 1000, 1200 and 1400 mg/kg body weight, a significant ($p < 0.05$) dose-dependent *in vivo* antimalarial effect were observed for suppressive, curative and prophylactic test respectively. Extract doses of 1000, 1200 and 1400 mg/kg/day, showed chemosuppressive activity of 66.47 %, 72.74 % and 80.33 % compared to 87.03 %, of chloroquine and 88.39 %, of artesunate respectively. The prophylactic test of the extract showed 66.57 %, 71.17 % and 75.41 % suppression, while 64.90 %, 78.01 % and 82.72 % suppression was observed for curative activities compared to 85.12 % and 91.68 % of chloroquine and 86.01 % and 93.83 % of artesunate respectively. The results confirmed the blood schizontocidal activity of methanol extract of *Salacia senegalensis* leaf at all phases of malarial infection (Adumanya *et al.*, 2014a). The comparative chemosuppressive effect of the extract vis-à-vis the standard drugs showed a dose dependent effect as shown in Table 1.

The highest suppressive effect was observed with the standard drug artesunate (88.39%) followed by chloroquine (87.03 %). The values were however comparable to that obtained for the extract dose at 1400 mg/kg/day which gave 80.33 % suppression. A dose dependent effect was observed on the comparative repository activity of the extract vis-à-vis chloroquine and artesunate used as shown in Table 2. When the extract dose increased from 1000 mg/kg/day to 1400 mg/kg/day (maximum dose used), repository activity increased from 66.57 % to 75.41 %, which is also comparable to 85.12 % obtained with chloroquine and 86.01 % obtained with artesunate respectively.

Table 1: Comparative **chemosuppressive activity** of chloroquine/artesunate and methanol extract of *Salacia senegalensis* leaf against *P. berghei* infection in albino mice.

Treatments	**Chemosuppressive** activity (% suppression of parasitaemia)
Normal Saline (5 ml/kg b.w)	0.00^{e} ±0.00
Extract (1000 mg/kg b.w)	66.47^{d} ±3.41
Extract (1200 mg/kg b.w)	72.74^{c} ±2.99
Extract (1400 mg/kg b.w)	80.33^{b} ± 3.23
Chloroquine (5 mg/kg b.w)	87.03^{a} ±2.87
Artesunate (3.2mg/kg day 1, 1.6mg/kg days 2-5)/kg b.w	88.39^{a} ±3.18

Means with different superscripts in the same column are significantly different from each other (P<0.05).Values are means ± standard deviation of five (5) replicates.

Table 2: Comparative repository activity (prophylactic effect) of chloroquine/artesunate and methanol extract of *Salacia senegalensis* leaf against *P. berghei* infection in albino mice.

Treatments	**Prophylactic** (Repository) activity (% suppression of parasitaemia)
Normal Saline (5 ml/kg b.w)	0.00^{e} ±0.00
Extract (1000 mg/kg b.w)	66.57^{d} ±4.72
Extract (1200 mg/kg b.w)	71.17^{c} ±5.96
Extract (1400 mg/kg b.w)	75.41^{b} ±6.99
Chloroquine (5 mg/kg b.w)	85.12^{a} ±5.49
Artesunate (3.2mg/kg day 1, 1.6mg/kg days 2-5)/kg b.w	86.01^{a} ±6.41

Means with different superscripts in the same column are significantly different from each other (P<0.05).Values are means ± standard deviation of five (5) replicates.

Also a dose dependent effect was observed with the comparative curative effect of chloroquine/artesunate and the plant extract as shown in Table 3.

Table 3: Comparative **curative activity** of chloroquine/artesunate and methanol extract of *Salacia senegalensis* leaf against *P. berghei* infection in albino mice.

Treatments	**Curative** activity (% suppression of parasitaemia)
Normal Saline (5 ml/kg b.w)	-
Extract (1000 mg/kg b.w)	64.90^{c} ±7.06
Extract (1200 mg/kg b.w)	78.01^{d}± 5.21
Extract (1400 mg/kg b.w)	82.72^{b} ± 3.63
Chloroquine (5 mg/kg b.w)	91.68^{a}± 2.16
Artesunate (3.2mg/kg day 1, 1.6mg/kg days 2-5)/kg b.w	93.83^{a}± 1.62

Means with different superscripts in the same column are significantly different from each other (P<0.05).Values are means ± standard deviation of six (6) replicates.

Highest curative activity was observed at 1400 mg/kg/b.w (maximum dose used) of the extract used. This gave 82.72 % curative activity compared with 91.68 % obtained for chloroquine and 93.83 % obtained for artesunate respectively.

Table 4: Comparative activity of antimalarial treatments on percentage mean survival time of the *P. berghei* infected mice and monitored over 30 days

TREATMENT	Percentage **(%)** mean survival time
Normal Saline (5ml/kg)	36.00
Salacia senegalensis (S.s.) 1000 mg/kg b.w	58.33
S.s 1200mg/kg b.w	68.89
S.s 1400 mg/kg b.w	80.57
Chloroquine mg/kg b.w	95.67
Artesunate (3.2mg/kg b.w day 1,1.6mg/kg days 2-5)	96.67

The better performance observed for artesunate and chloroquine compared with the extract in this study agreed with report by Kamei *et al.,* (2000), that when a standard antimalarial drug is used in the management of *Plasmodium berghei* in mice, it suppressed parasitaemia. This highest percentage chemosuppression activity of artesunate, followed by chloroquine recorded in the study showed that these drugs could still serve as antimalarial drugs (Borrmann, *et al.*, 2002; Fidock *et al.*, 2004; Hamedi, *et al.*, 2004; Haroon, *et al.*, 2005; Mejia Torres *et al*., 2013). Also the better performance observed for artesunate and chloroquine compared with the extract in this study agreed with the report that artesunate and chloroquine are still effective and still remains the traditional first line treatment for the treatment of malaria especially uncomplicated malaria (Kamei *et al.,* 2000; Kamya *et al*., 2001; Borrmann, *et al.*, 2002; Hamedi, *et al.*, 2004; Haroon, *et al.*, 2005; Yeshiwondim *et al.*, 2010; Mejia Torres *et al*., 2013). It also supports previous work on antimalarial activity of chloroquine by Oyewole *et al*., (2008) and Odeghe *et al.,* (2012) and artesunate (Mejia Torres *et al*., 2013).

The antimalarial property of this plant is as a result of phytochemicals (Adumanya *et al.*, 2014a). The percentage mean survival time of the *P. berghei* infected mice treated with extract doses were comparable to those treated with the standard drugs-chloroquine and artesunate as shown in Table 4. Therefore, the antimalarial activity/performance of the *Salacia senegalensis* extract observed was comparable to that of chloroquine and artesunate – drugs used as first line treatments for malaria.

Conclusion

The study showed that the antimalarial activity/performance of the methanol extract leaf of *Salacia senegalensis* is comparable to that of the two standard drugs (chloroquine and artesunate) used.

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Conflict of interest: None

Figure 1: *Salacia senegalensis*

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