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Full Length Research Paper

Evaluating the Antiplasmodial Activity of *Sphenocentrum jollyanum* Pierre Leaf and Root Extracts *In Vivo*

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Methanolic leaf and root extracts of *Sphenocentrum jollyanum* Pierre were tested *in vivo* for anti-malarial activity in Swiss albino mice inoculated with chloroquine resistant *Plasmodium berghei* NK 67 strain. The two extracts exhibited a significant (p>0.05) dose dependant anti- plasmodial activities in isolation and when combined in the 4-days curative standard test with a high mean of survival time. Although, the standard drug, Arthemether - lumefartrin, showed the highest antimalaria potency (81.4%), those of the leaf (74.4%) and root extracts (54.1%) in isolation and combination (63.4%) compared favorably. The leaf extract demonstrated higher antimalarial potency than the root or combination. The two extracts also produced a significant (p>0.05) positive effect on the weight and hematology values in the treated animals. Phytochemical screening of the plant (leaf and the root extracts) reveals the presences of flavonoids, steroids, terpenoids, tannins and alkaloids. The anti-plasmodia activity of these plant extract might be attributed to these phytochemicals compounds. The results showed that the leaf and root of S*. *jollyanum* possesses an anti-malarial activity which was effective against chloroquine resistance strain. This work has validated the traditional uses of *S. jollyanum* root extract in the treatment of malaria and also reported for the first time the anti-malarial property of the *S. jollyanun* leaf extract.

Key words: *Sphenocentrum jollyanum*, *Plasmodium berghei,* Arthemther lumefartrin, hematological parameter, anti-plasmodium.

INTRODUCTION

Malaria infection remains a major public health problem in the World. It is one of the most killer diseases world-wide with 90% of the death recorded in Sub-Saharan Africa (Mccombie, 2002). Control of the disease mostly relies on drug therapies but most currently available anti-malaria drugs have been used for decades with the rapid emergence of widespread drug resistance (Tona et al., 2001; Tolu et al., 2007; Ene et al., 2009). In spite of the socio economic problem and health hazard associated with poor treatment and management of malarial infections, most people in rural and urban area in Southwest Nigeria still relies on medicinal plants for the management and treatment of the disease. WHO, also reported that 80% of the population of Countries in Africa use traditional medicine to help meet some of their primary health care needs (WHO, 2001) and that the use of medicinal plants is spreading in popularity in industrialized countries (WHO, 2003).

Sphenocentrum jollyanum (S. jollyanum) root extract is one of the plants commonly used in the treatment of malaria infection in Southwestern part of Nigeria. *S. jollyanum* belongs to the family of Menispermaceae. It is a small erect sparsely branched shrub, growing up to 1.5 m in height. Traditional uses of different part of *S. jollyanum* in treatment of various ailments in West Africa Sub-region are common practice (Amidu et al., 2008; Moody et al., 2006). The roots are bright yellow with a sour taste when chewed (Neuwinger, 1996) and are used as 'chewsticks', relief for constipation, as a stomachic, as a cough medicine, for sickle cell disease, rheumatism inflammatory conditions, malaria treatment (Burkill, 1985; Iwu, 1993; Moody et al., 2006), as a central nervous system (CNS) stimulant and for anxiogenic and aphrodisiac in Ghana (Abbiw, 1990; Owiredu et al., 2007; Woode et al., 2009).

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Some scientific work have shown that the plant possesses antiviral and anti-inflammatory activities (Moody et al., 2002a, b, 2006), anti- oxidant and anti-angiogenic property (Nia et al., 2004). Recently, Raji et al. (2006) showed that methanol root extract of *S. jollyanum* increased the testosterone levels in albino rats and also caused a dose-dependent reduction in progressive motility of spermatozoa, viability and total sperm count. Though several studies have been carried out on pharmacological activities of the *S. jollyanum* plant extracts, to the best of our knowledge no scientific study has been recorded on the effect of the leaf or root extracts on plasmodium. Hence, an investigation on its anti-plasmodia activity will be of great importance and results of this work may open door for new drugs discovery.

MATERIALS AND METHODS

Plant material

Fresh *S. jollyanum* leaf and root were collected in November 2008 from University of Ibadan Botanical Garden and authenticated by Professor AO Adebisi of Department of Agricultural Science. A voucher specimen of the plant was deposited in the Faculty of Agricultural Sciences University of Ibadan, Ibadan. Oyo State, Nigeria.

Plant extracts

Dried and powdered leaves (500 g) and root (1 kg) *S. jollyanum* were separately and exhaustively macerated in 100% methanol for 72 h and filtered using a Buckner funnel and Whatman No 1 filter paper. The filtrate was concentrated under reduced pressure at 40ºC. 13.0 and 19.2% yield of extract was obtained for the leave and root extract, respectively. This was reconstituted separately in distilled water to give the required doses used in this study.

Phytochemical analysis

Phytochemical screening was carried out according to the methods described by Harbone (1973) and Trease and Evan (1989).

Animals

Swiss mice (13 to 17) g of both sex were obtained from Nigeria Institute of Medical Research (NIMR) Yaba, Lagos. Nigeria. The animals were housed in standard cages placed in well ventilated house conditions (temperature 22 ± 2°C: photoperiod: on 12 h light/dark cycle each throughout the experimental period; humidity: 55 to 69%). The animals were fed with pelletized grower mash from commercial feed vendor (Bova Jay Nig. Ltd, Ogbomoso) and water *ad libitum*. The experiment was carried out after its approval by the Ethics Committee of the Ladoke Akintola, University of Technology in accordance with the recommendations of the proper care and use of laboratory animals.

Malarial parasite

The chloroquine resistant *Plasmodium berghei* NK67 used for the study was obtained from the Nigeria Institute of Medical Research (NIMR) Yaba, Lagos. The parasite was maintained by sub-passaging into health mice every 2 weeks.

Hematological examination

Blood was collected by cardiac puncture from infected treated and untreated Swiss mice and was placed in 1 ml vacutainer EDTA anti-coagulated tubes for hematological analysis to determine blood picture profile. Hematological indices were evaluated as anemia and thrombcytopaemia are recognized features of malaria infection and to monitor disease progression (Chang and Stevenson, 2004; Moore et al., 2007)

In vivo antimalarial test

The *in vivo* anti-plasmodia activities of the leaf and root extracts of *S. jollyanum* were determined using a 4-day curative standard test described by David et al. (2004) and Peter and Anatoli (1998). The mice were divided randomly into groups of six mice each. Different doses of the leaf, root extract and their combination (50, 100 and 200 mg/kg) were orally administered respectively to mice in Groups 1 to 9. 5 mg/kg/day Arthemether and lumefartrin (Coartem) from Dannase pharmaceutical (positive control) and 10 ml/kg of distilled water (negative control) were respectively given to mice in Groups 10 and 11.

The blood from a donor mouse with 35% parasite was collected in a heparinized syringe. The parasitized blood was diluted in physiological saline solution (0.9% NaCl) to a density of 1×10^7 RBCs /ml. Extract treated groups and control mice were inoculated with parasitized blood on day 0. The extracts and drug were administered once daily for 4 days from day 3 of infection. Blood for film analysis on the third and seventh day were obtained and their body weight were recorded on daily basis and thereafter at weekly intervals over a period of 60 day. Films were Giemsa stained and examined microscopically to determine the levels of parasitemia. All mice were observed twice a day to assess their clinical signs, which were recorded. The animal experiments complied with all relevant guidelines and institutional policies on animal ethics.

Data and statistical analysis

Data are expressed as mean ± S.E.M. and analyzed using one way analysis of variance (ANOVA) followed by Dunnett test for comparing pairs of data. The significant level was set at $p < 0.05$.

RESULTS AND DISCUSSION

Anti-plasmodium *in vivo* test

The menace of multi-drugs resistant malaria parasite and the absence of a functional safe and widely available malaria vaccine have necessitates research in the direction of development of new antimalarial drugs. In this crusade, it is very important that chemical components derived from plants used in traditional medicine for treatment of malaria are investigated.

The results of the present study indicated that the methanolic leaf and root extracts of *S. jollyanum* or their combination produced a dose dependent chemo-suppressive antimalarial activity (Table 1). The highest parasite inhibition was observed at the dose of 200 mg/kg

Table 1. Effect of leaf and root extracts of *S. jollyanum* in isolation and combined on growth of *P.berghei* infected mice.

Extract/Drug	Dose (mg/kg)	Average (%)		NOD
		Parasitaemia	Inhibition (%)	
SJLE	50	6.88±0.03	43.97*	5
	100	4.30±0.02	64.98*	0
	200	3.10±0.02	74.75*	1
SJRE	50	6.88±0.03	43.97	2
	100	6.20±0.02	49.51	1
	200	6.63±0.02	54.15*	5
SJLRE	50	5.24±0.02	57.00*	2
	100	4.54±0.02	62.70*	1
	200	4.45±0.02	63.40*	0
COARTEM	5	2.88±0.01	81.43*	1
NEG-CON	DMSO	12.28±0.01	-	5

Data are expressed as mean ± SEM, SJLE, SJRE, SJLRE represent *S. jollyanum* leave, root, and combined; respectively, NOD, number of death recorded + positive control (Coartem) and NEG represent negative control. *p<0.05 when compared with control.

Table 2. Phytochemical constituents of methanolic leaf and root extracts of *S. jollyanum*.

Bioactive	Leave extract	Root extract
Flavonoid	+	+
Akaloids	+	+
Saponin	+	-
Glycoside	-	-
Tannin	+	
Terpenes	+	+.

+, Present; -, absent.

body weight of the mice. Percentage parasite inhibition of the leaf extract (74.8%) at 200 mg/kg on the 7th day is significantly ($P < 0.05$) higher than the root (54.2%) or the two extracts combined (63.4%). The orthodox anti-malarial drug, arthemether – lumefartrin (standard drug) which serve as positive control expectedly showed significantly ($p<0.05$) high anti-malarial potency (81.4%) on the 7th day after drug administration. Both extracts, especially the leaf extract by their percentage parasite inhibition even curative ability compared favorably with the arthemether – lumefartrin. Arthemether- lumefartin is an artemisinin-based combination therapies prequalified drugs use in countries experiencing high levels of antimalarial- drugs resistance parasite. The leaf extract appear to be more active than the root extract singly or when they are combined. The observed anti-plasmodia activity of the extracts may be due to the phytochemical compounds such as alkaloids, terpenes (monoterpenes), flavonoids and saponin (Table 2) which have been identified in this study (Christensen et al., 2001).

The numbers of dead mice as a result of the infection decreases with increase concentrations of the extracts when administered singly or combined. All mice treated with 50 mg/kg of the leaf extract died by 12th day, 2 died by 10th day in the group treated with 50 mg/kg of root extract and 2 died by 14th day in the group treated with 50 mg/kg of the combined extract (leaf and root). No death was recorded in the groups treated with 100 and 200 mg/kg concentration of leaf or combined extracts. However, all the animals in the group treated with 200 mg/kg of root extract died by the 9th day. All other mice survived up to day 60 without any sign of toxicity. The 100% death recorded on the 12th day in the group treated with 50 mg/kg of the leaf extract may be because of low concentration of the extract which is too small to cope with the burden of the infection, while toxicity of the root extract may be responsible for the death of the animals on the 8th day in the group treated with 200 mg/kg (Raji et al., 2006).

The numbers of death recorded in the other treated group may be as a result of poor animal handling or low amount of the extracts.

Effect of extracts on Hematological parameters and weight

Aneamia, hypoglycemia, body weight loss, and body temperature reduction are the general features of malaria-infected mice (Perlmann and Troye-Blomberg, 2007). Methanolic extract of *S. jollyanum* leaf (ESJL),

Table 3. Effect of methanolic leaf and root extracts of *S. jollyanum* on weight of *P. berghei* infected mice after 7 days of treatment.

Test substance	Dosage (mg/kg)	Average weight of mice		Change in weight (%)
		D_3	D_7	
Leaf extract	50	13.05 ± 0.01	13.65 ± 0.02	4.22*
	100	14.02 ± 0.03	14.97 ± 0.01	6.78*
	200	13.75 ± 0.02	15.03 ± 0.02	9.31*
Root extract	50	13.01 ± 0.03	13.44 ± 0.01	3.31*
	100	13.25 ± 0.01	13.98 ± 0.03	5.51*
	200	12.41 ± 0.02	13.40 ± 0.02	7.98*
Combined extract	50	12.02 ± 0.03	12.85 ± 0.04	6.91*
	100	13.04 ± 0.04	13.98 ±0.02	7.21*
	200	12.41 ± 0.02	13.40 ± 0.05	7.98*
Arthemether and lumefartrin	5	16.20 ± 0.01	17.47 ± 0.02	7.83*
Vehicle	1 ml	14. 21 ± 0.03	13.20 ± 0.02	- 7.11

Data are expressed as mean ± SEM for five animals per group. *p<0.05 when compared with control.

Table 4. Effect of the combined methanolic leaf and root extracts of *S. jollyanum* on hematological parameters and differential white blood cell count in *P. berghei* infected mice.

Parameter	Combine (200 mgkg^{-1})	PC (5 mg kg^{-1})	NC
PCV	44.0±0.02*	47.0±0.01*	37.0±0.01
MCV	87.0±0.01*	83.0±0.01	82.0±0.01
MCHC	34.0±0.001*	33.0±0.001*	29.0±0.001
HB	14.0±0.001*	15.0±0.01*	11.0±0.01
NEU	32.0±0.001	32.0±0.001	33.0±0.001
LYM	60.0±0.002*	53.0±0.001*	63.0±0.001
MONO	2.0±0.002	1.0±0.002	3.0±0.001

Data are expressed as mean± (SEM) for five animals per group * p<0.05. PC represents positive control, while NC represents negative control.

root (ESJR) and their combination (ESJLR) caused a significant ($p < 0.05$) weight gain in treated groups (Table 3). The comparison analysis indicated that the extracts significantly prevented weight loss in dose dependent manner compared to the negative control. The results showed that the physical status of the rats were better and this may be due to ameliorating effect of the plant extracts on acute fluid loss, proteolysis and lipolysis which are usually associated with weight loss in malaria infection (Jeremiah and Uko, 2007). Interaction study (ESJL and ESJR) on NK67 Chloroquine resistance *P. berghei* infected mice showed additive activities. The group treated with the combined extracts showed better survivability compared to groups treated with the leaf or root extracts singly. According to Chang and Stevenson (2004), malaria infection is associated with increased risk of severe anaemia. Taylor and Hurd (2001) revealed that the PCV of malaria parasite infected rodents as measured by haematocrit in the range of 43 to 44%, and this occurred approximately 48 h post-infection. The observed anaemia in *P. bergheim* infected mice may be due to RBC destruction caused either by parasite multiplication or by spleen reticuloendotelial cell action (Chinchilla et al., 1998). In this study, the extracts and their combination at concentration of 200 mg/kg produced a significant positive ($p < 0.01$) effect on the hematological (PCV or haematocrit), MCV, MCHC, hemoglobin (HB)) and white blood cells values except neutrophils and monocyte when compared with the negative control (Table 4). The slight increase in hemato-logical values demonstrated an improvement in disease progression (Chang and Stevenson, 2004; Piguet et al., 2002; Weatherall et al., 2002).

In conclusion, our study demonstrated that the methanolic extracts of the leave (ESJL) and root (ESJR) of *S. jollyanum* possessed significant anti-plasmodia

activity as seen in their ability to suppress *P. berghei* infection in mice and the leave is more potent than the root contrary to traditional believe. Further study is planned to determine the anti-plasmodia activity of the plant stem, fruit and to isolate the active compound(s) in the most active extracts. The mechanism of action of the most active compound will also be evaluated.

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