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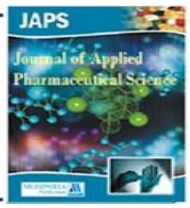

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Efficacy of *Thonningia Sanguinea* Vahl. (Balanophoraceae) Root Extract Against *Plasmodium Berghei, Plasmodium Chabaudi*, Inflammation and Nociception in Mice

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ABSTRACT

The effects of *Thonningea sanguinea* Vahl. root extracts were tested against *Plasmodium berghei* and *Plasmodium chabaudi*, acetic acid induced abdominal constriction and egg albumin induced paw oedema in rodents. Eighteen mice assigned to 3 groups of 6 animals each were infected with *P. berghei* (NK 65 chloroquine sensitive strain). Group I was treated with 300 mg/kg bw *T. sanguinea*, group II with 5mg/kg bw chloroquine phosphate (standard) and group III with 20ml/kg bw normal saline (control). Another set of eighteen mice were also inoculated with *P. chabaudi* and treated similarly. *P. berghei* was significantly suppressed by the extract over the time course of the study with mice survival periods of 36, 20 and 16 days for chloroquine, plant extract and normal saline treatments respectively. *T. sanguinea* produced some initial suppression of parasites but subsequently resurgence in parasitaemia was observed in the case of *P. chabaudi* infected animals. Mice survival periods with the later were 24 days (CQ), 22 days (extract) and 10 days (normal saline). Whole body weights significantly decreased in *P. chabaudi* but not *P. berghei* infected mice. Packed Cell Volume significantly ($p<0.05$) decreased with both models irrespective of the treatments. The extract had a minimal (10.89%) analgesic effect and had no anti-inflammatory activity. *T. sanguinea* though effective only in the *P. berghei* model could still be further investigated.

Keywords: *Thonningia sanguinea*, *Plasmodium berghei*, *Plasmodium chabaudi*, *Parasiteamia*, Resurgence,

INTRODUCTION

The plant kingdom is a source of a vast array of natural products that have been exploited as medicaments for a variety of disease conditions. These include analgesics, antipyretics (codeine, aspirin), antimalarials (quinine, artemisinin), emetics (ipecac), tranquilizers (reserpine), cardiac stimulants (digitoxin), anticancer (taxol, vincristine and vinblastine) and the AIDS products, (+) calanolide A and (-) calanolide B (Haidet, 2003). It has also been estimated that 80% of the world's population use herbal medicines (Adiukwu *et al.*, 2011; Jigam *et al.*, 2011[a]; Mukesh *et al*.,2010).

While some disease conditions are responsive to common and reliable therapeutics, malaria presents a frightening global statistic an estimated 300 million acute infections and up to 1 million deaths annually. These are primarily due to resistance by *plasmodium* parasites to common drugs and the absence of safe and viable vaccines (Anoka *et al.,* 2008; Jigam *et al.,* 2011[b]). The current trend is therefore to screen medicinal plants with reputations as antimalarials or fever remedy for bioactive principles against *Plasmodium* (Okunji *et al.,* 2000).

Thonningia sanguinea (Balanophoraceae) also called kulla by the Hausa of Nigeria is a strange subterranean plant devoid of chlorophylls with bright red coloured flowers which parasitizes *Hevea brasiliensis* (rubber tree), *Phoenix dactylifera* (oil palm) and Theobroma cacao (cocoa trees) (Idu *et al.,* 2011; Ryutaro *et al.,* 2011).

The flowers and rhizomes of the plant are used in herbal medicine as vermifuge, astringent, treatment for dysentery, diarrhea, leprosy, cutaneous infections, abscesses, dental caries, gingivitis, haemorrhoids and fever (Dalziel, 1955). *T. sanguinea* extracts have also been reported to posses antibacterial activity including multidrug resistant strains (N'guessan *et al.,* 2007). Etkin (1997) demonstrated the efficacy of the plant against *Plasmodium falciparum* in cell culture. These reports necessitated the invivo screening of *T. sanguinea* extracts against *P. berghei* and *P. chabaudi,* two commonly employed models of choice for many aspects of malaria research (Spence *et al.,* 2011; Jambou *et al.,* 2011).

MATERIALS AND METHODS

Plant Materials

Thonningia sanguinea rhizomes were obtained from herbal medicinal vendors in Minna Northern Nigeria and authenticated at the Department of Biological Sciences, Federal University of Technology, Minna.

Preparation of Crude Extracts

50g of air dried plant materials were micronized and extracted exhaustively (48 h) in the cold with 2L of methanol, (Sigma-Aldrich, Europe). The marc was filtered with muslin cloth and solvent removed under reduced pressure in a rotary evaporator. Brown coloured paste obtained was freeze dried and weighed prior to analysis.

Animals

Healthy swiss albino mice of either sex of about 6 weeks old weighing between 20 – 30 g each and wister rats of about 180 – 200 g weights obtained from National Institute of Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria were used for the experiments.

The rodents were conveniently housed under standard environmental conditions. (Temperature 27 ± 2°C; 70% relative humidity; 12hrs daylight/night cycle) and had free access to commercial feed pellets and water. Experiments were conducted in strict compliance with internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review (CCAC, 1997).

Parasites

P. berghei NK65 and *P. Chabaudi* chloroquine sensitive strains were obtained from NIPRD Abuja, Nigeria and maintained in our laboratory by serial passage in mice.

Safe dose and acute toxicity (LD_{50})

Five groups (A,B,C,D and E) of four mice each were used. The extract was dissolved in dimethylsulphoxide (DMSO) (Sigma chemicals; St. Louis, M. O. USA). The animals were given extracts intraperitoneally (i.p) at doses of 200, 400, 800, 1200 and 1600 mg/kg body weight (bw) in A,B,C,D and E respectively.
A control group was given normal saline (0.9% w/v NaCl) at 20 ml/kg bw. Mice were observed over 72h. Clinical signs and mortality were recorded. LD_{50} was obtained graphically as the intercept of % mortality (y-axis) and dosages (x-axis).

Antiplasmodial screening

Mice were pre-screened by microscopy of thin and thick tail tip blood smears. This was necessary to exclude the possibility of test animals harboring rodent *Plasmodium species.* The method by Fidock *et al*. (2004) was used. It involved the commencement of treatment on the third day post inoculation of mice with parasites. Eighteen male and female mice were divided into three groups of six each. A mouse infected with *P. berghei* (Parasitaemia of about 20 - 30%) was anaesthetized with chloroform and its blood collected by cardiac puncture with a sterile syringe and needle earlier flushed with heparin. The blood was diluted with normal saline such that 0.2 ml contained about 1 x 10^7 infected cells. Each of the eighteen clean mice were inoculated (i.p.) with 0.2 ml diluted blood. The extract at a dose level of 300mg/kg body weight was administered subcutaneously once daily for four days (D3, D4, D5 and D6). A parallel test with chloroquine (5 mg/kg bw) in the second group served as reference.

The third group was given normal saline and served as control. Thick and thin films were made from tail blood from D3 and alternate days up to D20, fixed with methanol and stained with 4% Giemsa (pH7.2) for 45 min before being examined under a microscope. Five fields were examined on each slide and the number of infected and uninfected red blood cells (RBC) counted and means taken. Mean parasitaemia per microscope filled (y-axis) was plotted against number of days (x-axis). *P. chabaudi* was similarly inoculated in another set of mice and the procedure repeated as in the case of *P. berghei* above.

Weight and Packed cell volume

Weights of mice infected with either *P. berghei* or *P. chabaudi* were taken using Avery Balance (W and T Avery Ltd, Birmingham, UK) over the duration the of analysis. Packed Cell Volume (PCV) was also determined for these groups of animals using the microhaematocrit method (Green, 1976).

Nociceptive (Analgesic) activity

Analgesia was assessed by the method of Koster *et al.* (1959). Fifteen mice were divided into 3 groups of 5 animals each. The extract (300mg/kg bw) was administered mice in group A, an hour before they were challenged with acetic acid (0.75% v/v). Animals in group B were however pretreated with Acetyl Salicylic acid (150 mg/kg bw) as reference drug, while group C which were given normal saline (20 ml/kg bw) served as controls. Five minutes elapsed before the numbers of abdominal constrictions induced by acetic acid were counted. Observations were made over ten minutes and mean value for each group calculated. Percentage inhibition of abdominal constriction by the plant extracts and ASA were determined in relation to the control.

Anti-inflammatory activity

The anti-inflammatory activity of the extract was tested using egg albumin induced paw oedema in rats (Winter *et al.*, 1962). 18 adult rats were divided six per each treatment group and used for the analysis. Inflammation was induced by the injection of 0.01 ml egg albumin into the sub-planter surface on the right hind paw 30 min after administering the extracts (300 mg/kg bw i.p). The increase in volume (cm^3) of the hind paw was measured with a LETICA digital Plethysmometer (LE 7500) before and at 20 min interval after the injection of egg albumin for a period of 2 hr. Control rats received an equivalent amount of normal saline while ASA (150 mg/kg bw) served as reference. The percentage inhibition of oedema was calculated for each dose.

Statistical Analysis

Results were expressed as mean ± standard error of the mean ($\overline{x}$ ±SEM). ANOVA was used to analyse data within groups and student's T-test to analyze data between groups. $P<0.05$ was taken as level of significance.

RESULTS AND DISCUSSION

The extract yield of *T. sanguinea* in methanol was 4.5g (11.25%), safe dose of the extract was determined as 300 mg/kg body weight with LD_{50} of 1450 mg/kg body weight of mice.

Antiplasmodial activity

The effects of *T. sanguinea* extract against *P. berghei* in mice is shown in Fig. 1. It indicates a progressive decline in parasitaemia from a peak of 86 parasites per microscope field on day three (3) down to sixteen (16) ppmf on day eighteen (18). Subsequently the parasite load remained uncleared between days eighteen (18) and twenty (20). On day fourteen (14) parasiteamia was totally cleared by the standard chloroquine treatment. However untreated animals (Normal saline) died on day fourteen. Survival periods were 36, 28 and 16 days for chloroquine, *T. sanguinea* and normal saline treatments respectively. Fig. 2 illustrates *P. chabaudi* response to *T. sanguinea* treatment. Parasitaemia declined initially but subsequently progressively increased from day six (6) to 115 ppmf on day twenty (20) leading to mortality in mice. The standard chloroquine treatment cleared *P. chabaudi* on day twenty (20). The normal saline treated mice (control) died on day twenty (12). Survival periods were 24 days (CQ), 22days (*T. sanguinea*) and 10 days (normal saline).

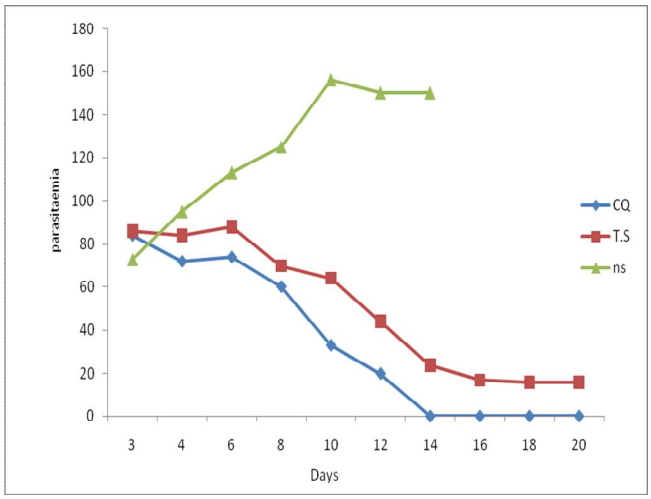

Fig.1: Activity of *Thonningia sanguinea* extract in *P. berghei* infected mice.

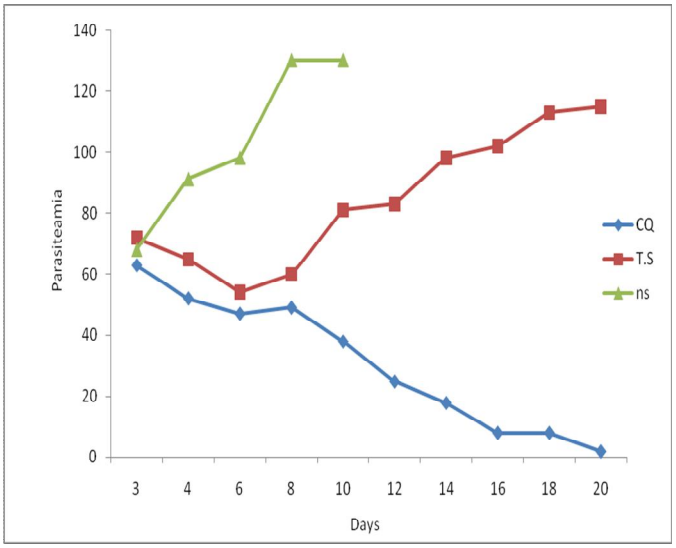

Fig.2: Activity of *Thonningia sanguinea* extract in *P. chabaudi* infected mice.

Whole body weight and PCV of mice

Mice infected with *P. berghei* (Table 1) and treated with *T. sanguinea* extract exhibited minimal variations in weight over the period of study. The weight ranged between 24.02±1.13g-23.08±0.06g (chloroquine); 25.11±1.34g-24.46+1.98g (*T. sanguinea*). However a significant depreciation (23.06±0.15g - 18.22±2.17g) was obtained with normal saline treatment. PCV declined significantly 37.35±1.80-33.22±0.06 (Chloroquine); 36.83±2.15 -28.50±1.30 *T. sanguinea*, 39.17±2.18 -18.46±0.25 normal saline. The case of *P chabaudi* (Table 2) infection whole body weight of mice significantly depreciated for chloroquine 26.04±1.53-19.16±1.44g, 27.08±2.35-18.03±1.91 (*T.sanguinea*), 25.57±1.10-16.88±0.02g (Normal saline). PCV ranges were 38.55±2.33-33.38±1.28 (chloroquine), 35.83 ±1.24-26.65±2.18 (*T. sanguinea*), and 32.00±2.36-19.05±1.31 (Normal saline).

Table. 1: Weight and PCV variations in *P. berghei* infected mice.

Treatment	(a) Weight (g) Initial	Final
Chloroquine	24.02± 1.13	23.08± 0.06
T. sanguinea	25.11± 1.34	24.46± 1.98
Normal saline	23.06± 0.15	18.22±2.17*
	(b) PCV	
Chloroquine	37.35±1.80	33.22±0.06*
T. sanguinea	36.83±2.15	28.50±1.30*
Normal saline	39.17±2.18	18.46±0.25*

n=6 *p<0.05

Table. 2: Weight and PCV variations in *P. chabaudi* infected mice.

Treatment	(a) Weight (g) Initial	Final
Chloroquine	26.04± 1.53	19.16± 1.44*****
T. sanguinea	27.08± 2.35	18.03± 1.91*
Normal saline	25.57± 1.10	16.88±0.02*****
	(b) PCV	
Chloroquine	38.55±2.33	33.38±1.28*
T. sanguinea	35.83±1.24	26.65±2.18*
Normal saline	32.00±2.36	19.05±1.31*

n=6 *P< 0.05

Nociception and Anti-inflammation.

Results of the analgesic (nociception) effect of *T. sanguinea* are in Table 3. It shows The plant extract had only 10.89 % analgesic activity while the standard acetylsalic acid treatment had 83.17%. T. sanguinea did not reduce paw oedema in rats hence had no anti-inflammatory effect. The standard acetylsalicylic acid treatment however had 72.84% anti-inflammatory activity.

Table. 3: Effects of *T. sanguinea* extract on acetic acid induced abdominal constriction in mice (analgesia).

Treatment	Dose (mg/kg bw)	Abdominal Constriction.	Inhibition (%)
Acetylsalicylic acid	300	45	83.17
T. sanguinea	150	8.5	10.89
Normal saline	20ml	51	--

n=5

Table. 4: Effects of *T. sanguinea* extract on rat paw oedema (anti-inflammation).

Treatments	Dose (mg/kg bw)	Paw Oedema (mm2)	Inhibition (%)
Acetylsalicylic acid	300	0.84	72.84
T. sanguinea	150	0.22	0.00
Normal saline	20ml	0.81	--

n=6

DISCUSSION

Thonningia sanguinea extract had a high safety margin of 300mg/kg body weight and LD_{50} of 1450mg/kg bw of mice. The absence of acute toxicity explains its wide spread consumption in folk medicine and as soup flavouring (N'guessan *et al*., 2007; Dalziel, 1955). The dietary use of antimalarial plants is pharmacologically advantageous as populations in endemic zones are exposed to constituents of potential therapeutic efficacy, thus having a compound effect of contributing to the low rates of malaria among such groups (Etkin, 1997). The significant suppression of *P. berghei* in mice by *T. sanguinea* extracts is a confirmation of earlier reports. The root extracts in ethanol, chloroform, sodium chloride, mixture of water and ethanol significantly inhibited *Plasmodium falciparum* in cell culture (Etkin, 1997). Literatures abound as to the antimicrobial activity of *T. sanguinea* extract. Some antibacterial agents have also been linked with antiplasmodial action (N'guessan *et al.*, 2007; Jigam *et al.*, 2011[a]). The better activity of *T. sanguinea* against *P. berghei* compared to *P. chabaudi* is noteworthy. This could be related to the mild clinical course of *P. berghe*i which is ideal for chemotherapeutic research especially when crude products are being tested. The more virulent nature of *P. chabaudi* is thus best suited for analysis of purified compounds as in drug resistance studies (Cox, 1998; Smyth, 1996; NIPRD, 2000). Recent pioneering studies on *P. berghei* and *P. chabaudi* could explain the disparity of response to crude products between the two species (Spence *et al.*, 2011; Jambou *et al*., 2011). The survival rate of mice infected with the two *plasmodium* species and treated with *T. sanguinea* was favorably comparable with standard chloroquine treatment. This was especially important in *P. chabaudi* infection. *T.sanguinea* has been reported to contain antioxidants e.g Thonningianins A and B, and also posses hepato protective action and potent antimicrobial effects (Gyamfi and Aniya 2001, Ohiri and Uzodinma 2000, Ohtani *et al., 2000*, N'guessan *et al*., 2007). These could explain the longer survival period of such mice despite the high parasitaemia noted in them. The significant decrease in whole body weight of mice and packed cell volume could be attributed to the presence of anti nutritive factors in the crude extracts. *Plasmodium* infection is correlated with the incidence of high destruction of red blood cells, hence anemia which could be life threatening (Jigam *et al*., 2011[b]). *T. sanguinea* extract had no anti-inflammatory activity and very low analgesic effect. These two pharmacological phenomena though desirable in an ideal anti malarial agent are not absolute requirements (NIPRD, 2000). This is more so that indigenous herbal medicines are often prepared as concoctions containing different plants acting synergistically to effect a common therapy (Etkin, 1997).

CONCLUSION

The use of *Thonningia sanguinea* in herbal medicine to treat malaria has been validated by the present study. It could thus be further analysed to identify its bioactive principle(s).

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