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Effects of *Mangifera indica* and *Casuarina equistifolia* extracts on survival and haematocrit of *Plasmodium berghei* infected mice

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Abstract
The mean survival time (mst) and haematocrit was determined following treatment of *Plasmodium berghei* infected mice with *Casuarina equistifolia* and *Mangifera indica* (leaf) extracts during established infection and repository test procedures. The survival time was significant (P<0.05) and dose dependent compared to the control group. The packed cell volume (PCV) taken on day zero and seven following established infection indicated that both extracts failed significantly (P<0.0.5) in preventing reduction of PCV in infected mice although some what lesser percentages where observed in 400 and 200mg/kg *C. equistifolia.* In the repository test, significant drop in PCV (P<0.05) was observed in all the groups except the chloroquine phosphate group.

Keywords: Mean survival time; PCV; *Casuarina equistifolia*; *Mangifera indica*; *Plasmodium berghei*

INTRODUCTION

The reduction of malaria attributable to mortality depends on accurate and early diagnosis followed by prompt treatment with effective drugs, the choice of which depends on knowledge of the antimalarial sensitivity profile of the local strains of *P. falciparum* (Adam *et al*., 2005) Furthermore, increased efforts in antimalarial discovery are urgently needed to develop safe and affordable new drugs to counter the spread of malaria parasites that are resistant to existing agents (Fidock *et al*., 2004). It has been discovered that the two most widely used antimalarial drugs, chloroquine (CQ) and sulphadoxine-pyrimethamine (SP-fansidar) are failing at an accelerating rate in most endemic regions, Nigeria inclusive, with consequent increases in malaria related-morbidity and mortality (Greenwood and Mutabingwa, 2002; Molta *et al*., 2004).

In recent years, natural products are of interest because drug resistance by diseases is on the increase (White and Nosten, 1993) and herbal remedies are being sought by a cross-section of scientists for various ailments (Odetola and Bassir, 1980). The use of herbs for disease management in Africa and Nigeria in particular could be traced to early man who probably acquired the skill of healing through deliberate or accidental selection of plants and their parts (Sofowora, 1982).

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Mangifera indica (Anacardacecae) and *Casuarina equistifolia* (Casuarinaceae) have differently been known to posses antimicrobial properties (Onwuliri and Umezurumba, 2003). Traditionally *M. indica* have been employed in the treatment of malaria in West Africa particularly south west Nigeria (Adesegun and Coker, 2001; Ajaiyeoba *et al*., 2004). In some parts of the world the plants have been employed in treatment of chills, fever, diarrhoea, skin rashes and sores (Coe and Anderson, 1996; Longuefosse and Nossin, 1996). We employed these plants to evaluate their anti-malarial properties as justification of their usefulness in traditional medicine. The preliminary reports on the aqueous extracts indicated activity against *P. berghei* in laboratory mice in all the three test models; suppressive, curative (established) or repository (Malann and Ajayi, 2008).

EXPERIMENTAL

Plant materials and extraction: The plants *C. equistifolia* Casuarinaceae (whistling pine leaves) and *M. indica* Anacardaceae (Mango leaves) were obtained from the school of forestry Jos. The leaves were spread thinly on a flat clean tray and air-dried at room temperature for seven days then reduced to coarse powder using a wooden pestle and mortar (Sofowora, 1982). Extraction was carried out as described by Epkendu *et al*. (2000). The powder (100g) each of the extracts were transferred into a conical flask and macerated in 300mls of distilled water. The mixture was allowed to stand overnight, then shaken for 3hours using a mechanical shaker. Filtration of the extract was through a Buckner flask using a suction/vacuum pump and the resulting filtrate was evaporated to dryness using a rotary evaporator.

Animals. Albino Swiss mice of weight between 20-22g of either sex where obtained from the Animal House Unit University of Jos. The animals were maintained under standard conditions at the Animal Facility Centre of the National Institute for Pharmaceutical Research and Development (NIPRD) Idu, Abuja. The animals were fed with standard diets and allowed access to water.

Parasite species and inoculation. Chloroquine sensitive NK65 *P. berghei* obtained from the National Institute for Pharmaceutical Research and Development (NIPRD) Abuja where a donor mouse with parasitaemia level of (++)=11-100 parasites per 100 thick film field. 1ml of blood was extracted through cardiac puncture using needle and syringe and made up to 20ml of physiological saline (Adzu *et al*., 2007).

Survival time: The mean survival time for each group was determined arithmetically by finding the average survival time (days) of the mice in each group over a period of 30days (day 0-30) post inoculation with *P. berghei*. After the establishment of parasitaemia on day four, treatment was initiated for 72 hours. The groups were orally administered *C. equistifolia* extract (400, 200 mg/kg/day), *M. Indica* extract (300, 200, 100mg/kg/day) chloroquine phosphate (5mg/kg/day) was given to the positive control group and 0.2ml of normal saline to the negative control group.

Determination of packed cell volume (PCV). The PCV was determined by the microhaematocrit method. On day zero heparinised capillary tubes were filled with blood from the tail of each mouse to about three quarters and immediately sealed at one end with crystoseal. The tubes were transferred to a microhaematocrit centrifuge and spun for eight minutes. The same procedure was repeated on day seven in curative (established infection) and repository test respectively. The capillary tubes were read using the microhaematocrit reader with its values in percentages. The repository test involves oral administration of all the animals with the extracts daily for 72 hours and on

day four the animals were inoculated with *P. berghei* and observed up to day seven (Adzu *et al*., 2007).

Statistical analysis. Each result is express as mean ± standard error of mean (SEM). The student t-test was use to compare results between treated and control groups for any significant difference. Values of $P \leq 0.05$ were considered significant.

RESULTS

The results indicated that the mean survival period was dose dependent for both plant extracts (Table 1). *M. indica* showed survival period of 25.6, 21.2 and 20.4 days for 300, 200 and 100mg/kg/day while *C. equistifolia* was 21.2 and 20.4 days for 400 and 200mg/kg/day respectively. Chloroquine 5mg/kg/day gave 28.4 days while the mean survival period for the untreated (control) group was 8.7 days.

Table 2 showed the analysis of the packed cell volume (PCV) on day seven. The extracts failed to significantly ($P<0.05$) prevent reduction in PCV, although lower percentages and significant preventive effects were observed compared to the control at 400 and 200mg/kg/day *C. equistifolia* extracts. The repository activity of the plant substances indicated a significant reduction in PCV on day four compared to the first day of parasite inoculation (Table, 3), only 5mg/kg/day chloroquine group prevented reduction in PCV on day four in contrast to that of the established infection procedure (Table 2). Similarly, there was less reduction in PCV on day four for 200mg/kg/day *M. indica* extract and 200mg/kg/day *C. equistifolia* extracts respectively. A high level reduction was observed in 200mg/kg/day *M. indica* extract in day four.

DISCUSSION

The preliminary photochemical screening and the acute toxicity study on *C. equistifolia* and *M. indica* extracts indicated the relative safety level of the plants extracts as well as the presence of the following metabolites; tannins, flavonoids, cardiac glycosides, steroids and carbohydrates.

Table 1: Mean survival time (mst) in days following established infection (Curative test).

Test Substance	Dose(mg/kg/day)	Survival (days)
M. indica	300	26.6±4.39
	200	21.2±5.38
	100	20.4±5.87
C. equistifolia	400	21.2± 5.42
	200	20.4±5.87
Chloroquine phosphate	5	28.4±1.67
Control (NS)	0.2ml	8.67±2.67

Each result is a mean of 5mice; NS = Normal Saline

Table 2: Packed cell volume test of *M. indica* and *C. equistifolia* leaf extracts against *P. berghei* in mice (curative test)

Test Substance	Dose (mg/kg/day)	PCV		% Reduction
		D 0	D7	
M. indica	300	45.00±1.52	42.80±2.71	4.89
	200	47.80±1.74	46.25±0.48	3.24
	100	49.00±0.89	48.00±3.76	2.04
C. equistifolia	400	43.00±1.39	42.75±1.32	0.58
	200	46.00±1.52	45.75±0.92	0.54
Chloroquine phosphate	5	45.20±0.86	43.80±2.94	3.10
Control (Normal Saline)	0.2ml	48.80±0.97	45.00±1.10	7.92

D - 0 = day infection was initiated D – 7 = 8th day of infection. Each result is with a mean of 5 mice.

Table3: Packed cell volume test of *M. indica* and *C. equistifolia* leaf extracts against *P. Berghei* (Repository test).

Test Substance	Dose (mg/kg/day)	PCV		% Reduction
		D 0	D7	
M. indica	300	44.60±1.81	36.00±9.02	19.28
	200	47.00±2.10	43.75±3.88	6.91
	100	44.60±1.95	39.00±5.51	12.56
C. equistifolia	400	46.20±1.39	40.50±2.40	12.34
	200	47.33±1.23	46.20±1.07	2.39
Chloroquine phosphate	5	44.60±1.33	47.00±1.08	-5.38
Control (Normal Saline)	0.2ml	47.20±1.32	42.75±2.29	9.43

D – 0 = day infection was initiated; D – 4 =4th day of infection; Each result is with a mean of 5 mice

In addition to these, *M. indica* extract contained alkaloids, saponins and anthraquinones (Malann and Ajayi, 2008).

Plants have been proven to be good sources of antimalarial agents especially with the success of quinine isolated from Peruvian cinchona bark and artemisinin from *Artemisia annua* (Adzu *et al*., 2007; Peters *et al*., 1986, 1993). The results of this study indicated that the mean survival time (mst) of the extracts treated groups were dose dependent and significantly different ($P<0.05$) compared to the control. The survival time of 300mg/kg/day *M. indica* was comparable to that of the standard drug (chloroquine phosphate group) which agrees to earlier findings on activities of *Crossopteryx febrifuga*, *Setaria megaphylla* and *Ziziphus spina-christi* (Elufioye and Agbedahunsi, 2004; Okokon *et al*., 2007; Adzu *et al*., 2007).

Packed cell volume is haematocrit value expressed as the percentage of cellular elements with that of whole blood. The determination of PCV helps in the diagnosis and treatment of anaemia (Sembuligam and Sembuligam, 2006). The haematocrit decreased markedly from day four following infection (Table 2 and 3) until death of animals as was observed in earlier works (Dikasso *et al*., 2006). This study indicated a percentage reduction in PCV with increase in parasitaemia. The control group had the highest percentage reduction of 7.92% compared to the other treated groups (Table, 2). This was also discovered earlier on (Dikasso *et al*., 2006). Similarly, reduction in PCV increased with increasing doses, although lesser percentage reduction in PCV was observed in the present study with the plant extracts and significant preventive effects were observed at some dose levels especially 400mg/kg/day and 200mg/kg/day *C. equistifolia* extracts (Table 2). In general, the effects were not consistent and conclusive since the haematological parameters to be evaluated in toxicity studies are on-going. The results also showed that it was only in one case of the repository studies that the reduction in PCV was halted when 5mg/kg/day chloroquine phosphate was used in treatment (Table 3).

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