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Research Article

Effect of Methanol Leaf Extract of *Nauclea latifolia* on Albino Mice Infected with *Plasmodium berghei berghei*

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Background: In Nigeria the leaf decoction of *Nauclea latifolia* is taken to treat malaria and sexually transmitted diseases. This study intends to generate a scientific data in support of the traditional use of the leaves in malaria treatment.

Objective: To investigate the antiplasmodial effect of the methanol extract of the leaves of *Nauclea latifolia* on chloroquine sensitive *Plasmodium berghei berghei* in experimentally infected albino mice.

Materials and Methods: The fresh leaves of *Nauclea latifolia* were collected, dried under shade, ground into powder and macerated in methanol for 72 hrs. The dried extract was stored at -4 °C for use. Thirty (30) mice were divided into five groups (A,B,C,D,E). Group A received 10 ml/kg/day of distilled water (negative control). Groups B, C and D received 370, 740 and 1110 mg/kg/day of the extract respectively. Group E received 1.2 mg/kg/day of artesunate (positive control). This experiment was repeated for suppressive, prophylactic and curative tests.

Results: The extract produced considerable antiplasmodial activity in all the three tests evaluated compared to the standard drug (artesunate). The extract reduced parasitaemia significantly ($p<0.05$) in a dose dependent manner. Bioactive constituents of the plant could be responsible for the antiplasmodial activity

Conclusion: The result of the study supports the need for continued search for components of traditional medicine as potential antimalarial agents.

Keywords: Malaria, *Nauclea latifolia*, mice, *Plasmodium berghei berghei*

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1. Introduction

There are about 380,000 species of plants in our world of which 260,000 belong to the higher plants; only 10% of the higher plants have been studied to a significant extent (Sandberg, 1976).

Nauclea latifolia is a multi-stemmed tree of up to 12 m tall. It has an open canopy. Flowers are joined by their calyxes. The fruit is a syncarp.

The young leaf contains mild alkaloids with analgesic property. The leaf preparations are taken as a vermifuge and diuretic, for amenorrhoea, antimalarial and sterility. It is hypotensive and hypoglycemic. In Corte de Voire, it is taken for jaundice and blood in urine, intestinal troubles and incipient hernia . In Ghana, it is taken for fever and jaundice. In Democratic Republic of Congo, leaf preparations are used externally on oedemas. The Igala of Northern Nigeria use a boiling water infusion of the leaf on swellings and skin rashes. The Tenda of South East Senegal crush and cook the

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leaves to make plasters for wound dressings. The leaves are deemed to have haemostatic property and are put on circumcision wounds and on haemorrhoids and hernias. The leaf extract is taken with chewed alligator pepper to relief malaria dysentery and diarrhoea. The leaf decoction is taken to treat sexually transmitted diseases (Burkill, 1997).

In a study, the aqueous extracts of *Nauclea latifolia* leaves (0.25-2.0 mg/ml) paralyzed *T. columbrifomis* larva in a concentration-dependent manner (Asuzu and Njoku, 1996). Ethanol stem extracts of *Nauclea latifolia* decreased the level of parasitaemia in a dose-dependent manner in mice experimentally infected with *Trypanosome brucei* (Madubunyi, 1995). In another study *Nauclea latifolia* extract was effective against nociception, inflammation and pyrexia in rats and mice thus partially justifying its use traditionally in the treatment of uncomplicated malaria and painful condition (Abbah et al, 2010).

Malaria is a mosquito-borne infectious disease of humans and other animals caused by protozoa of the genus Plasmodium (WHO, 2011). At present, 40% of the world population lives in malaria endemic regions (Snow, 2005). In 2001 about 700,000 lives of children below the age of five were lost to malaria in Africa. The disease is responsible for many medical complications like low birth weights in infants and increase in sudden abortion and stillbirth in pregnant women (Joda et al, 2005). Malaria is responsible for about 66% of all clinic visits in Nigeria. It causes the death of an estimated 250,000 children under the age of five every year. According to UNICEF findings, about 60% of deaths especially those of children in the Specialist Hospital in Bauchi, Nigeria are caused by malaria (UNICEF, 2009).

There is increase in the resistance of *P. falciparum* to antimalarial drugs. This development has compromised the efficacy of the available antimalarial drugs such as chloroquine, Fansidar and even artemisinin. Records have shown that there are no drugs that can offer protection against malaria in all the regions of the world (WHO, 2011).

The research on *Nauclea latifolia* reported herein therefore is a contribution to the research efforts towards identifying new potential treatments for malaria. Previous studies on *Nauclea latifolia* led to the development of a tablet dosage form from the water extract of this plant. The tablet produced had good mechanical properties like hardness but poor friability and disintegration (Emeje et al, 2005).

2. Materials and Methods

2.1 Collection of Plant Materials

The fresh leaves of *Nauclea latifola* were collected in September, 2011 from Ikot Ide village, Nto Edino Clan, in Obot Akara Local Government Area of Akwa Ibom State, Nigeria. The leaves were thoroughly washed with clean water and dried under shade for seven days to reduce moisture content and prevent enzyme action. The dry leaves were pulverized using mortar and pestle into powdered form and then preserved in a black air-tight polythene bag and stored away from light to prevent it from photolysis until required.

2.2 Extraction and Phytochemical Analysis

1.0 kg of the powdered leaves was macerated with methanol for 72 hr at room temperature (27±2 °C) with agitation 3 times daily to enhance the extraction process. The extracts were filtered out using filter paper (Whatman No. 1). The filtrates were evaporated to dryness using rotary evaporator and water bath at 40 °C. The concentrated extracts were then transferred to air-tight containers, corked and preserved in the refrigerator until required.

The methanol extracts were screened for phytochemicals using the standard methods of analysis by Trease and Evans (2009) and Sofowora (2008).

2.3 Breeding of Animals

Ninety (90) male albino mice weighing between 14-20 g were collected from the Animal House, Department of Pharmacology, University of Uyo, Nigeria. The mice were bred, food and water provided *ad libitum*. The animal house was cleaned and disinfected regularly and the sawdust changed three times a week.

2.4 Parasite Inoculation

A chloroquine sensitive strain of P. *berghei berghei* (ANKA) was obtained from the National Institute of Medical Research (NIMER), Lagos and was maintained by sub passage in mice (Basir et al, 2012).

Each mouse used in the experiment was inoculated intraperitoneally with 0.3 ml of infected blood containing about $1x10^7$ *Plasmodium berghei* parasitized erythrocytes. The inoculum consisted of $5x10^7$ *Plasmodium berghei* parasitized erythrocytes per ml. This was prepared by determining both the percentage parasitaemia and the erythrocytes count of the donor mouse and diluting the blood with isotonic (normal) saline in proportions indicated by both determinations: red blood counts and percentage parasitaemia (Odetola, 1980).

2.5 Evaluation of Antiplasmodial Activity

Evaluation of Prophylactic (Repository) Activities of Extracts

The repository activity of the extract was assessed using the method described by Peters (1965).

Thirty (30) mice were randomly divided into five groups of six mice each. Group A mice received 10 ml/kg of distilled water (negative control). Mice in groups B, C and D mice received 430, 860 and 1290 mg/kg body weight of the extract, respectively. Group E mice received 1.2 mg/kg of Artesunate (positive control). The treatment was carried out for three consecutive days (D_o – D_2) between 8 am and 9 am, using oral route of administration.

On the fourth day (D_3), the mice were inoculated with *Plasmodium berghei*. The parasitaemia level was assessed by blood smears seventy-two hours later.

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Evaluation of Suppressive Activity of the Methanol Extracts (4-Day Test)

The schizontocidal activity of the extract, distilled water and artesunate against early *P. berghei* infection in mice was evaluated using the method described by Knight and Peters (1980). Another thirty (30) mice were randomly divided into five groups of six mice each. On the first day, (D_o), the mice were infected with the parasite and randomly divided into various groups. These animals were given the extracts, distilled water and artesunate orally using oral carnula.

Group A mice received 10 ml/kg of distilled water (negative control); mice in groups B, C and D received 430, 860 and 1290 mg/kg body weight of the extract, respectively. Group E mice received 1.2 mg/kg of Artesunate (positive control). Dosing was done for four consecutive days (D_o – D_3) between 8 am and 9 am. On the fifth day, thick blood films were made from tail blood. The films were then stained with Leishman's stain to reveal parasitized erythrocytes out of 500 in a random field of the microscope. The average percentage suppressive effect of parasitaemia was calculated in comparison with the controls.

Evaluation of Curative Activities of Methanol Extracts (Rane's test)

Rane's test was used to evaluate the schizonticidal activity of the extract and artesunate in established infection. This was done as described by Ryley and Peters (1970). *P. berghei berghei* was injected intraperitoneally into another 30 mice on the first day (D_o). Seventy-two hours later (D_3), the mice were divided randomly into five groups of six mice each and administered orally.

Group A mice received 10ml/kg of distilled water (negative control); mice in groups B, C and D received 430, 860 and 1290 mg/kg body weight of the extract, respectively. Group E mice received 1.2mg/kg of Artesunate (positive control). Dosing was done for four consecutive days (D_o – D_3) between 8am and 9am. On the fifth day, thick blood film was made from tail blood. The film was then stained with Leishman's stain to reveal parasitized erythrocytes out of 500 in a random field of the microscope. The average percentage suppression of parasitaemia was calculated in comparison with the control.

2.6 Acute toxicity studies

Experimental Animals

Albino mice (30) of either sex weighing 16-23 g were obtained from the animal house of biological Science Department, University of Calabar and were housed in cages under standard condition (12 hours light and 12 hours dark), humidity and temperature in the University of Uyo animal house, Department of Pharmacology and Toxicology. They were fed on growers palletized feed and water.

Lethal dose Determination (LD_{50})

The up and down method (Bruce, 1985) was used to determine the toxicity level of the extract. The mice were fasted overnight and were grouped in threes. In the initial phase, four groups of the mice were administered with various doses of the crude methanol extract at the dose of 1.0, 2, 3.0 and 4.0g/kg body weight orally. The treated mice were monitored for 24hours for mortality and general behaviour. After 24hours, the remaining six groups of the mice were treated with the dose of 4.5, 4.6, 4.7, 4.8, 4.9 and 5.0g/kg body weight based on the findings of the phase 1 and were monitored for another 24hours (Lorke, 1983 and Bruce, 1985).

2.7 Ethical approval

Permission and approval for animal studies were obtained from the College of Health sciences animal ethics committee, University of Uyo.

3. Results

1.0 kg of the powdered leaves of *Nauclea latifolia* yielded 74 g (7.4%) of the methanol dried extract.

Phytochemical screening revealed that the methanol extract of the leaf of *Nauclea latifolia* contained alkaloids, cardiac glycosides, terpenes, polyphenols, saponins, tannins and flavonoids in high concentration, carbohydrates in moderate concentration and anthraquinones in low concentration. Cyanogenic glycosides, phlobatanins, balsams and resins were absent (**Table 1**).

Table 1: Phytochemical analysis of the methanol extract of *Nauclea latifolia* leaves

Constituent	Concentration in Leaf
Alkaloids	+++
Cardiac glycosisides	+++
Terpenes	+++
Cyanogenic glycosides	-
Carbohydrates	++
Anthraquinones	+
Polyphenols	+++
Saponins	+++
Tannins	+++
Flavonids	+++
Phlobatanins	-
Balsams	-
Resins	-

Key : +++ = *present in high concentration*
++ = *present in moderate concentration*
+ = *present in low concentration*
- = *absent*

The prophylactic / repository activity of methanol leaf extract of *Nauclea Latifolia* showed a dose–dependent reduction in parasitaemia in the extract treated groups (**Table 2**). These reductions were statistically significant relative to control ($p < 0.05$) but not as much

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as that exhibited by the standard drug artesunate at 1.2 mg/kg.

The chemosuppressive activity of methanol leaf extract of *Nauclea latrofolia* on parasitaemia increased as the dose of the extract increased (**Table 3**). These effects were statistically significant relative to control ($p < 0.05$) but not as much as that of the standard drug - artesunate

The mean survival time of mice receiving methanol leaf extract of *Nauclea latifolia* increased as the dose of the extract increased. The increases were statistically significant ($p < 0.05$) compared to control but not as much as that of the standard drug, artesunate (**Table 4**).

Table 2: Prophylactic/repository activity of methanol leaf extract of *Nauclea Latifolia* on *Plasmodium berghei berghei* infection in mice

Treatments	Group	Doses (mg/kg)	Parasitaemia	% chemosuppression
Distilled water (Negative control)	A	10 ml/kg	17.43±0.34	-
Extract	B	430	10.52±0.24	40.1
	C	860	8.24±0.28	53.0
	D	1290	6.21±0.44	64.4
Artesunate	E	1.2	3.01±0.06	85.2

*Values are expressed as mean ± SEM significance relative to control *p<0.05, (n=6)*

Table 3: Suppressive activity of methanol leaf extract of *Nauclea latrofolia* on *Plasmodium bergehei berghei* infection in mice 4 (day test)

Treatments	Group	Doses	Parasitameia	% Chemosuppression
Distilled water (Negative control)	A	10 ml/kg	22.02±1.05	-
	B	430	11.98 ±0.35	46.6
Extract	C	860	10.50±0.46	56.0
	D	1290	7.20 ±0.90	66.7
Artesunate (Positive control)	E	1.2	3.70±0.65	83.5

*Values are expressed as mean ± SEM significance relative to control * P<0.05, (n=6)*

Table 4: Mean survival time of mice receiving methanol leaf extract of *Nauclea latifolia* during established *P. berghei berghei* infection in mice

Treatments	Doses (mg/kg)	Mean survival time (days)
Normal saline	10 ml/ kg	10.36± 0.37
Extract	430	16.63 ± 0.74
	860	17.70 ± 0.35
	1290	22.33 ± 0.86
Artesunate (positive control)	1.2	26.28± 0.51

Values are expressed as mean ± SEM. Significance relative to control: P < 0.05

The maximum dose producing 0% mortality was 4.0 and the minimum dose producing 100% mortality was 4.7g/kg body weight. The LD_{50} was calculated to be 4.3 g/kg. The physical signs of toxicity observed included excitation, paw licking, increased respiratory rate, decreased motor activity, gasping, coma and death.

4 . Discussion

In traditional medicine the leaf of *Nauclea latifolia* has been used in the treatment of malaria, skin rashes, jaundice, fever, oedema, haemolysis, dysentery, hernias, diarrhoea and sexually transmitted diseases (Burkill, 1977). The folkloric use of this plant prompted the need

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to evaluate the in vivo antiplasmodial activity of its methanol leaf extract to provide some support for its ethnobotanical uses.

The extract reduced the parasitaemia in suppressive, prophylactic and curative models in a dose-dependent manner. These results may support the reported activity of the plant against norciception, inflammation, pyrexia which are symptoms uncomplicated malaria (Abbah et al, 2010)

Some secondary metabolites of plants such as alkaloids and terpenes have been associated with antiplasmodial activity (Ponarulselvam et al, 2012, Christensen and Kharazmi, 2001). In this study alkaloids and terpenes were present in abundance. Monoterpene indole-alkaloids have been implicated in endoperoxidation by generating reactive oxygen species which in turn may alkylate with heme protein resulting in the death of the parasite (Robert, 1998; Shigemori et al, 2003).

5. Conclusion

The Annang people in Akwa Ibom State, Nigeria drink the leaf decoction of *Nauclea latifolia* for prevention and cure of malaria. The result of this study has provided some scientific basis for its ethnomedical use and also supports the need for continued search for the active principle responsible for the antimalarial property for possible characterization.

Conflict of Interest declaration

The authors declare no conflict of interest

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