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Evaluation of the *in vivo* anti malarial activity of the methanolic leaf extract of *Nepata cateria*

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Nepata cateria **(Labiatae) growing widely in northern Nigeria is used by most indigenes for the treatment of malaria and other related diseases. The *in vivo* anti-malarial activity of the methanol leaf extract was evaluated in mice infected with the chloroquine sensitive *Plasmodium berghei berghei* NK65 strain. Oral acute toxicity of the methanol leaf extract with modified Lorke`s method was evaluated against early, established, curative, prophylactic, and residual infections and their mean survival period studied. The oral median lethal dose of the extract in mice was determined to be about 3800 mg kg^{-1} body weight. The extract at doses of 100, 200 and 400 mg kg^{-1} body weight produced significant ($P < 0.05$) dose dependent activity against the parasites in the suppressive, curative and prophylactic tests. The results suggested that the methanol leaf fraction of *N. cateria* possesses anti-malarial activity and thus lends credence to its ethno medical and folkloric usage as malaria cure.**

Key words: *Nepata cateria, in vivo, Plasmodium berghei berghei,* chloroquine, mice.

INTRODUCTION

Malaria is an ancient disease and has almost certainly caused more suffering and deaths than any other infectious disease, mostly in children under 5 years, especially in the developing world (Greenwood et al., 2005; Winter et al., 2006; WHO, 2008). This vector-borne infectious disease is a classical example of one that affects the productivity of individuals, families, and the whole society, since it causes more energy loss, more debilitation, more loss of work force, and more loss of economic/social damage than any other human parasitic diseases (Sachs and Malaney, 2002). We can easily understand why Riscoe et al. (2005) declared that "malaria has been responsible for the death of about half of all the people who ever lived". Taking cognizance of this disease and problems associated with anti-malarial drug resistance and prevalence of fake drugs in general circulations in the Nigerian markets, new drugs, drugs with fine-tuned modes of action or new drug combinations are urgently required hitherto for malaria treatment.

Plants have been a source of medicinal agents for thousands of years and an impressive number of modern

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drugs find their origin from it (Cragg and Newman, 2001; Brahmachari, 2006). Natural product chemistry has experienced an explosive and diversified growth in nature, making the subject of much interest and promise in the present day research directed towards drug design and drug discovery (Wessjohann et al., 2005). Thus the discovery of anti-malarial lead compounds is more than ever, a priority due to the alarming spread of resistance to available drugs and the limited number of effective anti-malarial drugs still in store (Peter and Antoli, 1998b). Recently, there has been a renewed interest in natural product research due to the failure of the alternative drug discovery methods to deliver many lead compounds in the key therapeutic areas such as immunosuppression, anti-infective and metabolic diseases (Henkel et al., 1999; Stalura et al., 2000; Lee and Schnieder, 2001; Feher and Schmidt, 2003). Natural product has played and will continue to play an invaluable role in the drug discovery process (Harvey, 2000; Newman et al., 2003).

The future of natural product in drug development thus appears to be a tale of justifiable hope. Faithful drives are needed in more intensified fashion to explore nature as a source of novel and active agents that will serve as leads and scaffold for elaboration into urgently needed efficacious drugs for a multitude of disease indications (Lee and Schnieder, 2001). Due to limited availability and/or affordability of pharmaceutical medicines in the tropical countries, majority of the populations depend on traditional medical remedies (WHO, 2002; Zirihi et al., 2005). *Nepata* species (Labiatae) are used in traditional medicine in many countries and have large ethnobotanical effects with diuretic, diaphoretic, vulnerary, antitussive, antispasmodic, antiasthmatic, tonic, febrifuge, emmenagogue and carminative properties (Nostro et al., 2001; Ghannadi et al., 2003). The Labiatae family contains 6,900 genera (Heywood et al., 2007) and 7,200 species, many of which are aromatic, including many widely used culinary herbs like basil, mint, rosemary, sage, savory, marjoram, oregano, thyme, lavender, and perilla.

Nepata cateria leaves are famous for inducing a delirious state in felines. Common names include cat wart, catmint, catnip (English), *Bunsurun fadama* (Hausa), *Kachikachiga* (Fulani) and *Wandin* (Tangale). Throughout history, this herb has been used in humans to produce a sedative effect (Tyler, 1994). Several disease conditions, including cancer, toothache, and corns have been treated traditionally with this plant. It is used traditionally to reduce gas and acts as a digestive (Sherry et al., 1979). It is used as a household herbal remedy, being employed especially in treating disorders of the digestive system, and stimulates sweating, thus reducing fever (Chevallier, 1996). The herb's pleasant taste and gentle action makes it suitable for treating cold, flu and fevers in children. A diethyl ether extract of *N. cateria* has been shown to have antimicrobial activity against fungi (*Aspergillus* and *Penicillium* spp.) and Gram-positive bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Salmonella enteritis* and *Pseudomonas aeruginosa*) (Antonia et al., 2001). The oil from *N. cateria* showed antibacterial activity against all the above strains of bacteria (Zenasni et al., 2008) but was more effective when used in conjunction with elder flower (*Sambucus nigra*) (Chievallier,). It is useful in the treatment of malaria, restlessness and nervousness in children (Grieve, 1984). A leaf infusion is also applied externally to bruises, especially black eyes (Genders, 1994). The major compounds found in *N. cateria* essential oil are nepetalactone, the stereoisomer 4a-α,7-α,7a-β-nepetalactone and dihydranepetalactone which represent more than 82% of the oil from the plant leaves (Zenansni et al., 2008). *N. cateria* also contains acetic, butyric, nepetalic and valeric acids, citral dipentene, citronellal, limonene, tannins, and volatile oils (HDW Inc., 2008).

The plant is said to deter insects such as mosquitoes, ants, fleas and beetles (Holtom and Hylton, 1979) as well as rats and mice from perching on them (Huxley, 1992). Here we report the oral acute toxicity of the methanol leaf extract with modified Lorke`s method against early malaria infection, curative effect against established infection and prophylactic effect against residual infection using chloroquine sensitive *Plasmodium berghei berghei* NK65 infected mice as our model organism.

MATERIALS AND METHODS

Collection of plant materials

Fresh sample of the leaves of *Nepata cateria* were collected in Gombe State University Campus Nigeria, on the 6th September, 2008. The samples were identified in the Biological Science Department of the University. The forestry herbarium index (FHI) number is 043 and a specimen of the plant was deposited in their herbarium. The samples (1.2 kg) was air dried in the Chemistry laboratory of the University before pounding to a fine powder using pestle and mortar to about 70 mesh size and then stored in a dry container

Extraction

500 g of the powdered sample was accurately weighed and percolated with 3.5 L of 85% methanol for 72 h, after which there was decantation, filtration and concentration using rotary evaporator (R110) at 35°C to obtain methanol soluble fraction, labeled, F_M^F (weight of 87.64 g). This fraction was heated over a water bath at reduced temperature to remove the remaining solvent and then stored in a refrigerator at -4°C for the tests.

Animals

The animals used in the work were four (4) weeks old-albino mice

weighing 18 to 22 g, obtained from the National Veterinary Institute Vom, Jos Plateau State, Nigeria, carried in cages to International Institute for Pharmaceutical Research and Development (NIPRD), Idu Abuja, Nigeria, where they were housed in plastic cages with saw dust as beddings and given food and water *ad libitum*. They were used in accordance with National Institutes of Health (NIH) guide for the care and use of laboratory animals NIH Publication (No. 83 -23) revised (1985).

Acute toxicity test (LD_{50})

The oral acute toxicity of *N. cateria* was assessed in mice using modified Lorke's assay (1983). The study was carried out in two phases. In phase one, nine mice were randomized into three groups of three mice each and were given 10, 100 and 1000 mg kg^{-1} body weight (b. wt) of the extract orally. The mice were observed for paw licking, salivation, stretching of the entire body, weakness, sleep, respiratory distress, coma and death in the first 4 h and subsequently daily for 7 days. In phase two, another fresh set of nine mice were randomized into three groups of three mice each and were given 1600, 2900 and 5000 mg kg^{-1} b. wt of the extract orally, on the result of the first phase. They were observed for signs of toxicity and mortality for the first critical 4 h and thereafter, daily for 7 days. The LD_{50} was then calculated as the square root of the product of the lowest lethal dose and highest non-lethal dose. The geometric mean of the consecutive doses for which 0 and 100% survival rates were recorded in the second phase, the oral median lethal dose was calculated using the formula:

LD_{50} =√ Minimum toxic dose (2900) × maximum toxic dose (5000)

or

Oral median lethal (LD_{50}) dose = $\sqrt{2900x5000}$ = 3800 mg kg^{-1}

Rodent parasite (*Plasmodium berghei berghei*)

The rodent parasite *P. berghei berghei* NK 65 was obtained from the National Institute for Medical Research (NIMR) Lagos, Nigeria. The parasites were kept alive by continuous intraperitoneal passage in mice (Adzu and Haruna, 2007) every 24 days. These infected mice were used for the study. Prior to the beginning of the study, one of the infected mice was kept and observed to reproduce disease symptoms similar to human infection (English, 1996).

Antimalarial studies

Suppressive test

The Peter`s 4 days suppressive test against chloroquine sensitive *P. berghei berghei* NK 65 infection in mice was employed (Peter, 1967). Adult Swiss albino mice weighing 18 to 22 g were infected by intraperitoneal (IP) injection with standard inoculums of *P. berghei berghei* with 1 × 10^7 infected erythrocytes. The mice were randomly divided into five (5) groups of six (6) mice per group and treated orally for 4 consecutive days with 100, 200 and 400 mg extract kg^{-1} b. wt. daily. Two control groups were used: Positive control was treated daily with 5 mg chloroquine kg^{-1} b. wt while the negative control was given 5 ml kg^{-1} normal saline. On day 5 of the experiment, blood was collected from the tail of each mouse and smear made (Saidu et al., 2000). The blood films were fixed with methanol, stained with 10% Giemsa at pH 7.2 for 10 min and parasitaemia determined microscopically. The percentage suppression of parasitaemia was calculated for each dose level by comparing the parasitaemia in infected control with those of treated mice. The average % suppression was calculated as:

$$\text{Average \% suppression} = \frac{A-B}{A} \times 100$$

Where A = Average percentage parasitaemia in negative control group, and B = Average percentage parasitaemia in test group.

Evaluation of Schizontocidal activity of N. cateria on established infection (curative or rane test)

Evaluation of the potential of the methanol extract of leaves of *N. cateria* was carried out according to the method described by Ryley and Peters (1970). The mice were infected intraperitoneally with standard inoculums of 1 × 10^7 *P. berghei berghei* NK 65 infected erythrocytes on the first day (day 0). Seventy-two hours later, the mice were divided into 5 groups of six mice each. The groups were orally treated with *N. cateria* leaves extracts at doses of 100, 200 and 400 mg kg^{-1} day^{-1}. Chloroquine was given as positive control (5 mg kg^{-1} day^{-1}) and an equal volume of distilled water was given to the negative control group. The treatment was carried out once daily for 5 days and tail blood smears were collected and examined microscopically to monitor the parasitaemia level.

Evaluation of the prophylactic activity of Nepata cateria (repository test)

Evaluation of the prophylactic potential of extracts of *N. cateria* leaves was carried out according to the method of Peters (1967). Adult mice were randomized into 5 groups of six mice each. Group 1 was given 10 ml distilled water kg^{-1} b. wt. orally. Group 2, 3 and 4 were orally given 100, 200, and 400 mg extract kg^{-1} b. wt. Group 5 was given 5 mg chloroquine kg^{-1} b. wt intraperitoneally. Treatments were initiated on day 0 and continued till day 4 when, the mice were all infected with the parasite. Blood smears were then made from each mouse 72 h after infection (Abatan and Makinde, 1986) and increase or decrease in parasiteamia determined as before.

Statistical analysis

The one way ANOVA test was used to analyze and compare the results at a 95% confident level, values of $P \geq 0.05$ were considered significant, results were expressed as Mean ± standard error (SE) of mean.

RESULTS AND DISCUSSION

Anti-plasmodial investigations

The antimalarial activity of the *N. cateria* fraction against *P. berghei berghei* NK65 is shown in Tables 1 to 5). Table 6 expresses the mean survival time. Thus, Oral median Lethal (LD_{50}) dose = √Minimum toxic dose × maximum toxic dose = √2900 × 5000 = 3800 mg kg^{-1}

Table 1. Acute toxicity test of the methanol extract of leaves *of Nepata cateria* (Phase I).

Concentration/wt. of mice 10 mg/kg (1 mg/ml)	Vol. (ml)	Signs of toxicity	Survival
18 g	0.18	x	1
19 g	0.19	x	1
22 g	0.22	x	1 (All 3 survived)
100 mg/kg (10 mg/ml)			
19 g	0.19	x	1
21 g	0.21	x	1
20 g	0.20	x	1 (All 3 survived)
1000 mg/kg (100 mg/ml)			
22 g	0.22	x	1
18 g	0.18	x	1
20 g	0.20	x	1 (All 3 survived)

x = No sign of toxicity.

Table 2. Acute toxicity test of the methanol extract of leaves *of Nepata cateria*. Phase II (Concentrations based on phase I)

Concentration/wt. of mice 1600 mg/kg	Vol. (ml)	Signs of toxicity	Survival
21 g	0.21	Paw licking	1
18 g	0.18	Paw licking	1
20 g	0.20	Stretching	1 (All 3 survived)
2900 mg/kg			
20 g	0.20	Salivation (spit)	1
18 g	0.18	Paw licking	1
18 g	0.18	Salivation	1 (All 3 survived)
5000 mg/kg			
19 g	0.19	Sleep	1
22 g	0.22	Comatose (tired)	1
20 g	0.20	Weakness	1 (All 3 died)

2900 mg/kg = minimum toxic dose and 5000 mg/kg = maximum toxic dose.

Antimalarial activity

Tables 2, 3, 4 and 5 express the results of the suppressive, curative, prophylactic and the mean survival periods, respectively, of Swiss albino mice treated with methanol leaf extract of *N. cateria* and chloroquine in established malaria infection.

Acute toxicity

The mice were treated orally with single dose each of 10 to 5000 mg kg^{-1} b. wt. of *N. cateria* leaves extracts after being starved for 24 h. The route was chosen because of its sensitivity and rapid results. The extract at 10 to 1000 mg kg^{-1} (phase 1) produced no physical signs of toxicity in

Table 3. Suppressive effect of methanol leaf extract of *Nepeta cateria*.and chloroquine against *P. berghei berghei* NK 65 infection in Swiss albino mice.

Treatment	Parasitemia (%)	% Chemo-suppression
Normal saline 5 ml kg^{-1} (control)	5.72±1.21	-
Extract 100 mg kg^{-1}	3.22±1.32*	56.04
Extract 200 mg kg^{-1}	2.34±0.93*	60.64
Extract 400 mg kg^{-1}	1.23±0.88**	73.20
CQ 5 mg kg^{-1}	0.42±0.27**	94.00

*Significant different from control at p≤ 0.05 and **at p≤ 0.01.

Table 4. Curative effect of *N. cateria* methanol leaf extract and chloroquine against *P. berghei berghei* NK65 infection in Swiss albino mice.

Treatment	Parasitemia (%)	% Chemo-suppression
Normal saline 5 ml kg^{-1} (control)	48.60±2.22	-
Extract 100 mg kg^{-1}	12.50±2.02*	62.53
Extract 200 mg kg^{-1}	4.20±0.62*	80.70
Extract 400 mg kg^{-1}	0.36±0.12**	89.19
CQ 5 mg kg^{-1}	0.21±0.14**	99.59

*Significant different from control at p≤ 0.05 and **at p≤ 0.01.

Table 5. Prophylactic effect of *N. cataria* methanol leaf extract and chloroquine against *P. berghei berghei* NK65 infection in Swiss albino mice

Treatment	Parasitemia (%)	% Chemo-suppression
Normal saline 5 ml kg^{-1}(control)	7.89±1.41	-
Extract 100 mg kg^{-1}	4.12±1.32*	34.21
Extract 200 mg kg^{-1}	2.44±0.8*	43.27
Extract 400 mg kg^{-1}	1.61±0.96**	55.48
CQ 5 mg kg^{-1}	0.62±0.32**	89.41

*Significant different from control at p ≤ 0.05 and **at p≤ 0.01.

Table 6. Mean survival period of Swiss albino mice treated with methanol leaf extract of *N. cateria* and chloroquine in established malaria infection.

Dose of extract (mg/kg/day)	Survival time (days)
Norman saline 5 ml kg^{-1}(control)	09
Extract 100 mg kg^{-1}	19
Extract 200 mg kg^{-1}	20
Extract 400 mg kg^{-1}	23
CQ 5 mg kg^{-1}	30

the mice 24 h after administration. But from 1600 to 5000 mg kg^{-1} (phase 2) there were some physical signs: salivation, paw licking, stretching/writing, calmness etc, within the first minutes of administration. There was however no mortality at all dose levels used. The median lethal dose LD_{50} was estimated to be ≥ 3800 mg kg^{-1} b. wt. However, the observed reduced activity of the treated mice showed that the extract possess central depressant

effect. The absence of death following oral administration of the extract, at below 5000 mg extract kg^{-1} b. wt. observed in mice suggested that the extracts were practically non-toxic acutely (Salawu et al., 2009). This high safety profile of anti-malaria efficacy was in human than in rodent models, the later have also been validated through the identification of several conventional anti-malaria drugs such as chloroquine, halofantrine, mefloquine, maldox and more recently artemisinin derivatives (Ryley and Peters, 1970)

Suppressive test

N. cateria leave extract exerted dose dependent chemo-suppressive effect against *P. berghei berghei* NK 65 malaria parasite. The extract caused a significant ($P < 0.05$) chemo-suppression of 56.04, 60.64 and 73.20%, when compared to the control. The standard drug chloroquine caused chemo-suppressions of 94.0% (Table 3) which was higher than the groups treated with the plant extract. The observed higher efficacy of chloroquine may in part be due to non selectivity of the extract or slow absorption and poor bioavailability of the crude extract. This is common with medicinal plants extracts (Adzu and Haruna, 2007). The significant chemo-suppression produced by the extracts on day 5 is consistent with the traditional use of the plant as an herbal medicament against malaria in Northern Nigeria.

Curative effect

N. Cateria leaf extract produced significant ($P < 0.05$) dose dependent reduction in parasitaemia levels in the extract treated groups of *P. berghei berghei* NK 65 malaria parasite with a almost 100% reduction as in the chloroquine treated group (positive control). The average percentage parasitaemia reduction of the extract treated groups on day 7 were 62.53, 80.70, 89.19% for the 100, 200 and 400 mg/kg/day. Chloroquine 5 mg/kg b. wt exerted 99.59% reduction of the parasite (Table 4). While there was a daily increase in the parasitaemia in the negative control group, the average percentage prasitaemia decreases in the extract and the positive control.

Prophylactic effect

The methanolic extract leaves of *N. cataria* produced significant ($P < 0.05$) dose dependent reduction in parasitaemia levels in the extract treated groups of *P. berghei berghei* NK 65 malaria of 32.21, 43.27 and 55.48% while 5 mg chloroquine kg^{-1} b. wt. caused 89.40% reduction in parasitemia (Table 5). The result indicated that the leaf extract of *N. cateria* possesses blood schizontcidal activity as evident from the chemo-suppression obtained during the five day early infection test and the 7 days each for curative/established infection which is comparable to the standard drug chloroquine (5 mg/kg/day).

Survival period

From (Table 6), the extract appears to be highly effective against the species of *P. berghei berghei (*NK 65). The mean survival period of the Swiss albino mice treated with the extracts in established infection during a period of one month (30 days) showed that as the dose increases, the survival time increases. Mice treated with chloroquine 5 mg/kg b. wt. per day survived for 30 days. Those treated with the extract at 100, 200 and 400 mg/kg b. wt. per day survived for 19, 20 and 23 days, respectively. The animals in the negative control group, which were treated with distilled water/normal saline, were found to have a mean survival period of 9 days. *P. berghei berghei* NK 65 parasite is used in predicting the treatment outcomes of any suspected anti-malaria agent due to its high sensitivity to chloroquine, making it the appropriate parasite for this research (Peter and Anatoli, 1998a). However, present findings seem to deviate from the previous study by Oze et al. (2007) who demonstrated that some plants may be nephrotoxic when administered in higher doses.

Currently, no single drug is effective for the treatment of multidrug resistant malaria and combination therapy includes artemisinin derivatives such as artesunate (David et al., 2004) or mixtures with older drugs such as atovaquone (Deprez-Poulain and Melnyk, 2005), proguanil (Jones and Good, 2006) combination malarone/maldox (Winter et al., 2006; Taylor and white, 2004). Unfortunately, first report on drug resistance to arteminin-derivatives (Jambou et al., 2005) and to drug combination therapies (Wichmann et al., 2004) have already appeared. So, in the absence of a functional, safe and widely available malaria vaccine, efforts to develop new anti-malaria drugs continues.

Conclusion

There is a consensus among the scientific community that natural products have been playing a dominant role in the discovery of leads for the development of drugs for the treatment of human diseases (Newmann et al., 2003). Indeed, the vast majority of the existing anti-malaria chemotherapeutic agents are based on natural products and this fact anticipates that new anti-malaria may

constantly emerge from our tropical plants sources if well harnessed, since biological chemo diversity continue to be an important source of molecular templates in the search for anti-malaria drugs (Portet et al., 2007). Further research to isolate, identify, elucidate and characterize the active ingredients, especially from the alkaloids, flavonoids, terpenoids and quinones from which most anti-malaria drugs have been isolated, are encouraged.

Conflicts of interest

Authors have none to declare.

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