Prophylatic Potential of Lemon Grass and Neem as Antimalarial Agents

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Abstract: Prophylactic activities of methanol, ethanol and aqueous extracts of neem and lemon grass against plasmodium development in mice were investigated. Various extracts of the plants were prepared with soxhlet apparatus. Growth and reproduction of malarial parasite in the treated animals was delayed 3 days after treatment. The mean % parasitaemia obtained in mice administered with methanolic, ethanolic and aqueous extracts of lemon grasss were 43.01%, 50.21% and 48.08% while those treated with methanol, ethanol and aqueous extracts of neem displayed 59.54%, 61.50% and 13.4% respectively indicating the anti – plasmodial activity of both plants. . It is therefore, concluded that the activities of these plants depend neither on weight of the mice nor dosage but on the solvent used. The parasitaemia development in the group treated with standard drug (Malariech) was significantly minimal having 2.47% and 88.23% % parasitamia and average % suppression recorded. Aqueous Neem extract exhibited highest suppressive effect 76.21% followed by Lemon grass in respect of the methanolic(43.67%) and aqueous(38.07%) extracts as compared with methanolic(25.47%) and ethanolic(23.32%) extracts of Neem.. The suppressive value of aqueous neem extract 76.21%, was considered significant and could serve as sufficient replacement for conventional antimalarial drugs that easily loose their potency with the impending development of resistance. [Journal of American Science 2010;6(8):503-507]. (ISSN: 1545-1003).

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1. Introduction

Before the advent of orthodox medicine in the treatment of ailments which include malaria, the traditional African society had devised various means of combating such ailments. One of the major ailments that is of concern in the world today is malaria. Malaria is the single most important cause of ill health, death and poverty in the Sub-Saharan Africa. About 200 to 300 million new cases of malaria occur worldwide each year, and about one to three million deaths occur of which 2 / 5 occur in Africa (WHO, 1999). The disease is believed to be a major obstruction to social and economic development in Africa, causing enormous misery and suffering through the pain of fevers and the anguish of bereavement. Ninety percent of the deaths caused by malaria occur in children aged less than five year old (WHO, 2004)

However, the problems militating against the effective management of malaria have been enumerated. The most important problem is that *Plasmodial* parasites are resistant to most widely available, affordable and safest first line treatments such as Chloroquine and Fansidar(Kisame,2005; Sendegive et al 2005) or secondly , the overall control of mosquitoes which transmits malaria is made difficult by their resistance to a wide range of insecticides. The third which is a new and rapid developing problem is the wide production of fake antimalarial drugs. In Southeast Asia 32% and 53% of artesunate blister packs sampled contained no active ingredients (Newton et al, 2006). Lastly, most countries in Africa lack the necessary infrastructure and resources to manage and control malaria (WHO, 2004).

Most times, accessibility and the affordability to health facilities and drugs are not within the reach of most people as a result of the location of the health centers and cost of antimalarial drugs. This could be seen as a factor to high death rates recorded in malarial cases. As a viable remedy, there is a continuous search for alternative low cost drugs and herbal preparations to replace those that lost their activities to drug resistance. A number of traditional herbs have been tested and used in the prevention and also treatment of malaria including *Artemisia annua* (Akininyi *et a*l, 1986), old leaves of *Carica papaya*, roots and leaves of *Guinensis*, unripe fruit of *Capsicum frutescence* and *Azadirachta indica* popularly called Dangoyaro in Nigeria. Recent studies indicate that lemon grass can be successfully used to treat drug resistant malarial and typhoid fever (Madu, 2007). It has been discovered that several drugs most of which are used for the treatment of

malaria can be taken for prevention. This study is aimed at determining the prophylactic effect of neem and lemon grass in malarial cases in order to ascertain the extent of the effectiveness of these plants as antimalarial agents and to verify the activities of the extracts from different solvent against malarial *Plasmodium.*

2. Materials and Methods

(a).Preparation of plant materials/ extractions and drug suspension

The leaves of *Cymbopogon citratus*(lemon grass) and *Azadirachta indica*(neem)were collected from and around Army barracks, Bori Camp Port Harcourt and Federal University of Technology, Owerri(FUTO). They were then taken to the laboratory of the Department of Pharmacology, College of Health Sciences, University of Lagos. The leaves were sorted to remove insects, variegated leaves and debris. They were then rinsed in water to remove dust particles after which they were allowed to air dry, with further drying in a Gallenkam oven at 40^0C for 6 hours, the leaves were then powdered using a blender for 15 minutes.

Methanolic, ethanolic and aqueous extraction of the lemon grass and Neem leaves were carried out by using soxhlet apparatus. Six extracts were obtained which were then heated to dryness using hot water bath. The concentrated extracts were scraped out with spatula. 500mg of each of the concentrates was weighed, then dissolved in 10ml of water and poured into sample bottles. Also, six tablets of malariech were dissolved in 10% sugar solution according to Isah *et al*, (2003). Both preparations were stored at 4^0C in the refrigerator in the laboratory till use.

(b). Sample Animal and weight determination

Swiss albino mice used in this research were collected from the Department of Pharmacology College of Health Sciences, University of Lagos and then transferred to the laboratory in the Department of Parasitology, Nigeria Institute of Medical Research (NIMR) Yaba, Lagos. A total of 37 mice were used, 22 female and 15 male mice weighing between 11g and 28g were fed on standard diets manufactured by Pfizer Livestock Feeds LTD, Nigeria. The same environmental conditions of 12 hours light and 12 hours darkness, normal room temperature of 27^0C were maintained for all the animals throughout the period of investigation. They were separated into 8 groups according to their weights as follows: 4, 6, 6, 6, 5, 5, 3, and 3 in each 3.5cm x 6.5cm cage.

(c). Malaria Parasite

Plasmodium berghei obtained from the Department of Parasitology. Nigeria Institute of Medical Research (NIMR), Yaba, Lagos was used.

(d). Standard Drug - Malariech

This drug was obtained from Maryland Pharmacy, Surelere, Lagos Nigeria

(e). Dosage determination

The dosage of each extract was determined according to Marcia *et al* (1992)

(f). Administration of Extracts

The drugs were administered to all the mice except those in group 8 by forceful feeding using an oral canular for 7 consecutive days.

Group 1 received methanolic extract of lemon grass
Group2 received ethanolic extact of lemon grass
Group3 received aqueous extractof lemon grass
Group4 received methanolic extract of neem
Group 5 received ethanolic extract of neem
Group 6 received aqueous extracts of neem
Group 7 received a standard antimalarial drug – Malariech
Group 8 received no drugs and served as control group.

All the animals were left for eight days after treatment, and then infected with *P. berghei* and left for three days after which thick blood smear was prepared from their tail veins.

(g). Inoculation of the mice

Each of the Swiss albino mice was intraperitonally administered with a standard inoculum of *P berghei*. Stored parasitized blood in liquid Nitrogen was allowed to thaw. The content was injected into 3 donor mice and left for five days so as to allow for the development of parasitaemia. The parasitized blood was collected from each donor mouse and diluted appropriately with Phosphate buffer saline to make 1×10^7 parasitized red blood cells, as described by Isah *et al* (2003). 0.2ml of the diluted inoculum was then injected into each of the sample mouse after which they were left for 3 days. The aim was to achieve a high level of parasitaemia. However, thick blood smear were prepared from their tail veins and viewed under the × 40 microscope after five days. The parasitaemia count was carried out by the use of a tally counter and the average percentage parasitaemia calculated using the approach of Obih and Makinde (1985) as thus:

Average % Parasitaemia = No of parasitaemia / No of WBC × 100.

Average % Suppression =
Av. % Parasitaemia in control − Av. % Parasitaemia in treated /Av. % Parasitaemia in Control × 100

3. Result

Parasite development was observed only in group 8 that received no drugs, with the average mean parasitaemia of 75.09% which increased to 90.94% 6 days after (Table 1&5). Since there was no development of parasitaemia in group 1- 7 after 3 day's infection, blood samples were then collected from each mouse, and smear made to check for parasite development in day 6. The mean % parasitaemia obtained in Gp1- 3 mice administered with methanolic, ethanolic and aqueous extracts of lemon grasss were 43.01%, 50.21% and 48.08% while in Gp 4 - 6, 59.54%, 61.50% and 13.4% for the methanol, ethanol and aqueous extracts of neem respectively(Table 2&3).The parasitaemia development in group 7 treated with standard drug(Malariech) was signicantly minimal with 2.47s and 88.23% as% mean parasitaemia average suppression recorded Aqueous Neem extract exhibited the highest suppressive effect 76.21% followed by Lemon grass in respect of the methanolic(43.67%) and aqueous(38.07%) extracts as compared with methanolic(25.47%) and ethanolic(23.32%) extracts of Neem(Table 6). The growth and reproduction of malarial parasite was evidently delayed after 72 hours for groups 1-7 which received treatment indicating the prophylactic activities from the extracts of the both plants

Table 1: Paristaemia count in group 8 mice 3 days after infection

Sample	Parasite	WBC	Av. % Parasitaemia
1	161	201	80.09
2	139	203	68.67
3	153	200	76.50

Table 2: Parasitaemia count in groups 1, 2 and 3 mice that were administered with methanolic, ethanolic and aqueous extracts of lemon grass after 6 days of infection

Since there was no development of parasitaemia in other groups after three days of passaging, blood samples were collected from each mouse and smear were made on the sixth day to check for parasite development.

solvent	Sample No	Average weight (g)	Average dose/ day	Parasite count	WBC	Average % Parasitaemia
Methanol / GP 1	1	11	0.3	91	203	44.8
	2	11	0.3	82	201	40.7
	3	11	0.3	77	200	38.5
	4	11	0.3	98	204	48.0
Ethanol / Gp2	1	15	0.4	102	200	51.0
	2	15	0.4	99	201	49.4
	3	15	0.4	107	201	53.0
	4	15	0.4	100	202	49.5
	5	15	0.4	97	200	48.5
	6	15	0.4	102	205	49.8
Aqueous / Gp3	1	15	0.4	98	200	49.0
	2	15	0.4	100	200	50.0
	3	15	0.4	99	201	49.3
	4	15	0.4	97	200	46.1
	5	15	0.4	94	202	46.3
	6	15	0.4	98	205	47.8

Table 3: Parasitaemia count in group 4 – 6 mice administered with methanolic, ethanolic and aqueous extracts of Neem after 6 days of Infection

Solvent	Sample No	Average weight(g)	Average dose/ day	Parasite count	WBC	Average% Parasitaemia
Methanol / GP 4	1	20	0.53	127	205	61.8
	2	20	0.53	115	202	56.1
	3	20	0.53	120	202	60.0
	4	20	0.53	122	203	60.0
	5	20	0.53	125	207	59.8
Ethanol / Gp5	1	20	0.53	150	208	72.1
	2	20	0.53	131	203	63.9
	3	20	0.53	123	207	59.4
	4	20	0.53	147	201	43.1
	5	20	0.53	139	202	68.1
Aqueous / Gp6	1	24	0.64	20	202	9.0
	2	25	0.64	25	201	12.4
	3	24	0.64	41	205	20.0
	4	24	0.64	30	203	14.8
	5	24	0.64	22	203	10.8

Table 4: Parasitaemia count in group 7 mice treated with standard drug (Malariech) after 6 days

Sample No	Average weight	Dose/day	Parasites	WBC	Average % parasitaemia
1	28	0.74	--	201	
2	28	0.74	15	202	7.4
3	28	0.74	—	200	

Table 5: Parastaemia count in group 8 in the Control group after 6 days of infection

Sample No	Average weight	Parasites	WBC	Average % parasitaemia
1	28	191	205	93.17
2	28	179	201	89.05
3	28	183	202	90.59

Table 6: Average % Suppression observed at 8 days after infection

Group	Average Suppression
1	43.67
2	35.74
3	38.07
4	25.47
5	23.32
6	76.21
7	88.23
8	—

4. Discussion

The extracts of the two plants, *Cympobogo citratus* and *Azadirachta indica* showed a high activity similar to results of Iwalewa et al (1992). From the results, the growth and reproduction of malarial parasite in the treated animals was delayed after 3 days. It showed that the pretreatment of mice with the extracts interrupted the establishment of parasitaemia against the control.

There were observed variations in the degree of prophylactic activities of the extracts with regards to the solvent used. Aqueous extract of Neem exhibited highest prophylactic effects (13.40%) on the development of malarial parasites followed by the methanolic and aqueous extracts of lemon grass that showed 43.20% and 48.09% prophylactic effect on mice 6 days after pretreatment. On the other hand, methanol extracted more active ingredients than ethanol; this may be due to the higher volatility of methanol than ethanol. According to Dutta (1993), different solvents dissolve different active compounds from the same plants. The aqueous extracts of neem had the greatest suppression / reduced parasitaemia value of 76.21% while methanolic extracts of neem had the least suppressive effect (23.32%) indicating that water extract of neem the most effective (Obaseki and Jegede, 1986). Although previous studies have shown that water extract of neem are less active than leaf extract obtained by water/ acetone combination (Udeinya, 1993). This result also corroborates Devi *et al* (2001) who reported that the aqueous extract of leaves of *Azadirachta indica* showed significant activity at 125 – 500mg/kg against *P. berghei.* . It is concluded that the activities of these plants depend neither on weight nor dosage but on the solvent used.

The average % parasitaemia by group 7 that received the standard drug (Malareich) was 2.47%, showing little parasite development. Also the average suppressive effect of neem from all the groups that were administered with neem was 47.10% while that of lemon grass extract was 44.81%, making the standard drug the most suppressive drug at a percentage of 96.71%. Although, the orthodox antimalarial drugs prove most effective but with regards to numerous problems associated with management of malaria such as the development of resistance to most drugs as well as substandard antimalarial drugs, coupled with fact that these drugs are neither affordable nor available to the reach of common man, use of these plant extracts should encouraged. Again, with 76.21% as the suppressive value of aqueous neem extract, definitely means that this plant could serve as sufficient replacement for conventional antimalarial drugs that may loose their potentials with the impending development of resistance. Obih and Makinde(1985) showed neem extracts to be effective even against chloroquine resistant strains sof the malarial parasite.

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