East African Medical Journal Vol. 79 No. 7 July 2002
PROPHYLACTIC EFFECT OF MULTI-HERBAL EXTRACT 'AGBO-IBA' ON MALARIA INDUCED IN MICE
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ABSTRACT

Objective: **To determine the efficacy of a multi-herbal preparation extract of 'Agbo-Iba' on rodent malaria induced in mice.**

Design: **An experimental design in which mice were divided into four groups A,B,C,D representing control, prophylactic, chloroquine and 'Agbo-Iba' groups respectively. Each mouse was intraperitoneally inoculated with *Plasmodium yoelii nigeriensis* and treated with oral herbal extract or chloroquine syrup depending on group.**

Setting: **College of Medicine of the University of Lagos Medical Microbiology and Parasitology Laboratory.**

Subjects: **One hundred and twenty male and female albino mice aged 10-12 weeks with an average weight of 25 grams.**

Main Outcome measures: **The herbal extract was effective, preventing the development of parasitaemia in the prophylactic group of mice.**

Results: **After intraperitoneal inoculation of *Plasmodium yoelii nigeriensis*, a prepatent period of two days was observed before parasitaemia was established in all but the prophylactic group of mice. Induced infection was promptly aborted with oral chloroquine treatment in group C, while in groups A and D, infection terminated fatally. Group B mice appeared normal throughout the duration of investigation with 100% survival rate.**

Conclusion: **'Agbo-Iba' extract has some prophylactic action against malaria induced in mice with no apparent significant side effects.**

INTRODUCTION

The use of herbal remedies in the treatment of diseases is universal and traceable to the ancient time when man acquired the skill of herbal healing through deliberate selection of plants or by accidental discovery(1,2). Thus, in Nigeria a cross section of both rural and urban dwellers, literate or illiterate, relies heavily on herbal preparations for the treatment of fevers suspected to be due to malaria, despite the availability of orthodox anti-malaria drugs(3). Presently, the most commonly used variety of herbal remedy, is 'Agbo-Iba'. This is decoction of a number of herbal plants used in the treatment of malaria fever, mostly in the Western part of Nigeria, with an increasing demand. The upsurge in the use of this remedy is attributable to:

(i) the claim of herbalists that 'Agbo-Iba' is more efficacious than the orthodox anti-malaria drugs
(ii) the emergence of resistant strains of *Plasmodium falciparum*, and
(iii) the escalating cost of treatment, the high incidence of adverse reactions and the poor safety margin (especially in pregnancy) associated with the use of orthodox anti-malaria drugs.

Ekanem(4) reported the suppressive effect of the aquous extract of *Azadirachta indica*, a commonly used herbal remedy, on mice induced malaria, while odetola and Bassir(5), also noted a suppressive action of Morinda lucide extract against *Plasmodium gallinaceum* in chicks. Some of the local plant materials employed in the preparation of 'Agbo-Iba' e.g. *Nauclea latifolia, Uvaria chmae, Cymbopgon gianganteas*, have been found to contain bioactive substances like alkaloids, saponnins, tannins, anthraquinone and phlobatannins, also claimed to be active against bacteria, fungi and other infectious agents(7-9).

Several documented reports abound which favour the use of herbal remedies in the treatment of malaria, based on various experimental models but using a single herbal extract(4-6,10). Therefore, this experimental study, using multi-herbal preparation 'Agbo-Iba' was undertaken as a preliminary step to ascertain the prophylactic potential of 'Agbo-Iba' in the local management of malaria.

MATERIALS AND METHODS

Plant Materials: All the plant materials used were collected from Mushin market in Lagos, Nigeria and duly identified in the department of Pharmacognosy of the College of Medicine, University of Lagos.

The preparation consisted of the following plants:

Ewe Otili	*Cajanus cagan* (leaf)
Ewe Opiri	*Euphorbia lateriflora* (leaf);
Ewe Mangoro	*Magnifera indica* (leaf)
Ewe Asuwon	*Cassia alata* (leaf);
Ewe Koo'ko-Oba	*Cymbopgon giganteas* (leaf)
Ewe Egbesi	*Nuclea latifolia* (leaf);
Epo Eeruju	*Uvaria chmae* (bark) and
Epo Mangoro	*Magnifera indica* (bark)

A total of two kilogrammes of plant materials comprising 250g of each plant were cut into smaller pieces, washed and boiled in two litres of water for three hours as specified by the herbalist. The filterate was stored at 4°C while not in use.

Parasites: Chloroquine sensitive strains of *Plasmodium yoelii nigeriensis* experimentally maintained by blood passages in Albino mice from the Department of Medical Microbiology and Parasitology of the College of Medicine, University of Lagos, were used.

Albino mice: Sixty male and 60 female albino mice (10-12 weeks old), with an average weight of 25 grams, were obtained from the College of Medicine, University of Lagos Animal House. They were fed on Rat and Mouse pellets, (manufactured by Pfizer Nig. Ltd. given clean water and kept in clean cages.

Drug: Chloroquine syrup containing 16mg/ml chloroquine sulphate was obtained from the Lagos University Teaching Hospital Pharmacy.

Inoculum: The inoculum which contained 2,725 *Plasmodium yoelii nigeriensis* parasitised red blood cells per 0.5ml in saline preparation was inoculated intraperitoneally into each mouse. The parasite concentration was determined by:

a. *Parasite count:* This was based on the number of parasitised red blood cells in 1000 red blood cells of donor mouse, obtained by counting under the light microscope, from a Giemsa stained thin film. The donor mouse parasitaemia was 20%.

b. *Volume of inoculation:* 0.05ml donor blood was added to 9.95ml of sterile physiological saline. 0.5ml of this saline preparation was inoculated intraperitoneally into all the mice.

c. *R.B.C Count:* 0.05ml donor blood was added into 9.95ml of Dacies fluid, from where the R.B.C. count was calculated, using Neubaeur counting chamber as follows:

Total R.B.C. counted = 2,725
Dilution factor = 200
Volume factor = 10
Total R.B.C. per ml = Total R.B.C. count x Dil. factor x vol. factor
= 2,725 x 200 x 10
= 545 x 10^4 R.B.C./cm^3

1ml donor blood contained 545 x 10^4 /cm^3 RBC.

∴ 0.05ml donor blood contained 545 x 10^4 x 0.05 RBC = 272500 RBC

Thus, 10ml saline preparation contained 272,500 RBC

Hence, 0.5ml saline contained $\frac{272500 \times 0.5}{10}$ = 13,625 RBC

Parasitaemia of donor blood = 20%.

∴ No of parasites inoculated = 20% of 13,625
= 2,725 parasite/mouse.

Method:

Administration of Drugs: The 120 mice employed in this experiment were divided into four groups as follows:

A – Control group 24 mice
B – Prophylactic group 32 mice
C – Chloroquine group 32 mice
D – Herbal preparation group 32 mice

Every mouse in each group was inoculated with 2,725 parasites intraperitoneally, using aseptic technique.

While the mice in group A were not treated, those in group B commenced drinking the herbal preparation extract freely as the only source of water. An average of 12ml of extract was taken by each mouse during the experimental period. Groups C and D mice were not treated until parasitaemia was established 48 hours after inoculation.

Thereafter, Group C mice were treated with 0.5ml chloroquine syrup containing 8mg base chloroquine sulphate, orally per mouse, once daily for three days, while group D

Table 1

Effects of chemotherapy on experimentally induced malaria in Albino mice

Experimental Animal Group	No.	Inoculum/ mouse	Prepatent period (days)	No. of Deaths after Chloro-quine or or herbal extract administration	Mortality after Chloroquine or herbal administration (%)
A	24	2,725	2	0	0
B	32	2,725	0	0	0
C	32	2,725	2	5	15
D	32	2,725	2	4	12
Total	120	10,900	-	9	27

mice received 0.5ml herbal extract orally once daily for three days.

The oral drugs were administered with blunt G19 needle and syringe which facilitated the placement of chloroquine and herbal extract into the stomach of group C and D mice respectively, as described by Oyerinde(8).

Course of Infection: The course of induced infection was monitored by daily examination of ciemsa stained blood film obtained from the tail of each mouse. This was continued until the parasitaemia cleared or the mouse died.

RESULTS

Five mice died from group C after oral administration of chloroquine, while four mice died from group D after receiving oral herbal extract (Table 1). This occurred within 1-3 hours of drug administration, giving a mortality rate of 15% and 12% respectively.

A prepatent period of two days was recorded for mice in groups A, C and D while in group B (those treated with the herbal extract) infection was not established and all animals in the group survived.

Among group A mice parasitaemia rose from 2.5% on the 3rd day to 55% on the 9th day when all mice in this group died. In group C mice, the established infection was aborted after three days of administration of 0.5ml chloroquine once daily. Among group D mice, parasitaemia increased significantly ($P < 0.01$) from 2% on the 3rd day to 59%, resulting in the death of all the mice (Table 2).

DISCUSSION

The effect of different treatment on various groups of mice experimentally infected with malaria is striking. The prepatent period in the groups where parasitaemia was established (i.e group A, C and D) was two days which conformed with the known property of the strain of *Plasmodium* employed in this investigation(11,12). In group A, daily parasitaemia followed the usual course in mice infected with *Plasmodium yoelii nigeriensis* where no treatment was given, resulting in the death of all the mice in this group eight days post-infection(13). This demonstrated the virulence of the plasmodial strain and the suitability of the mice used as induced infection is difficult to achieve in old mice(14).

The failure to establish parasitaemia in group B mice, after inoculation, suggests that the herbal preparation extract which the mice in this group drank freely (about 12ml/mouse), was enough to prevent infection and had served as a prophylactic remedy. Furthermore, that no death occured in this group suggests that the herbal preparation was well tolerated with no observable side effects during the study period of 36 days.

The administration of chloroquine sulphate to group C mice aborted infection within four days. This indicates that the parasite strain was sensitive to chloroquine and that the dose, though theoretically high, was tolerated as parasitaemia was below 4%(13-15). The pattern of infection in group D mice, which showed the longest course and highest degree of parasitaemia was similar to that of the control group (group A). It would appear that the amount of herbal preparation given to this group was not enough to stop infection or even reduce the level of parasitaemia, hence, the herbal preparation was not therapeutic at this dosage as the concentration of the active ingredients were not pre-determined.

The results obtained in this study (15% and 12%) were similar to those of Oyerinde(11) who reported

Table 2

Post infection daily parasitaemia (%) among Albino mice which received different treatment

Days after Infection	Parasitaemia (%)			
	Group A	Group B	Group C	Group D
1	0	0	0	0
2	0	0	0	0
3	2.5	0	2	2
4	12	0	3.5	11.5
5	24	0	3	22.5
6	34	0	1.5	30
7	48	0	0	40
8	55	0	0	45
9	DM	0	0	59
10	DM	0	0	DM

Key:
0 = No parasitaemia recorded
DM = Death of all mice in the group

15% and 17% mortality under similar experimental conditions. This confirmed earlier suggestion(11) that the procedure adopted in drug administration on the mice probably traumatised the internal organs of mice leading to death, although, other possible factors need be examined.

This investigation has demonstrated that the multi-herbal preparation 'Agbo-Iba' has prophylactic effect against *Plasmodium yoelii nigeriensis* in mice. Similar results had been reported by Onabanjo(10), who observed 60% recovery rate in mice infected with *Plasmodium berghei* and treated with root and leaf extract of *Morinda morindoides*. It is also worthy to note that inspite of the number of the different components of the herbal extract, no significant side effects were observed. This finding supports documented reports from other workers(16-18).

Therefore, it would appear that 'Agbo-Iba' has prophylactic potential at the dosage used herein. However, further studies are required to determine the active ingredients and the therapeutic dosage in both animals and humans.

ACKNOWLEDGEMENT

To Dr. A. Adeoye of the Department of Pharmacognosy, College of Medicine, University of Lagos for the identification of the herbs, Dr. (Mrs.) A.F. Fagbenro-Beyioku for her contribution, Mr. E. Isele for technical assistance and Mrs. C.B. Adeboye for secretarial services.

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