Therapeutic Potentials of *Azadirachta indica* and *Ocimum gratissimum*, and their Synagistic Effects in Treatment of *Plasmodium berghei* Infected Albino Mice

ABSTRACT

Antimalarial resistance is a major challenge for effective control of malaria. This triggered the need to monitor the efficacy of *Azadirachta indica* and *Ocimum gratissimum* in the disease management. The present study compared antimalarial activities of the leaf extracts of the individual plants and evaluated their combined effects on malaria infected mice. The leaves of the different plants were extracted with absolute ethanol (BDL 95%) for the test. Clean albino mice were experimentally infected intraperitoneally with chloroquine-sensitive *Plasmodium berghei* Nk65 strain. Parasitaemia level was determined before parasite inoculation and at 24 hours post treatment period. Efficacy of the leaf extracts was tested on the infected mice using Peter's 4-days suppressive and curative tests, and secondary biological assessment procedures.The lethal median dose (LD_{50}) recorded for neem and clove basil leaf extracts were 31.62 and 1246.9 mg/kg body weight, respectively. Infected mice treated with leaf extracts of the plants and their combinations produced significant dose-dependent activity against the parasite ($P<0.05$). Highest reduction of parasitaemia was observed on day 4. Maximum parasitaemia reduction (78.65%) was attained with 30mg/kg of the combination of the extracts on the 7th day. These observations indicate better anti-malarial activity of the combination therapy as compared with the individual extracts of the plants, and also show their good antimalarial potentials.

Keywords: Malaria treatment; drug resistance; phytochemicals; leaf extraction; synergism of phytochemicals.

1. INTRODUCTION

"Malaria is an infectious and life-threatening disease caused by *Plasmodium* species and vectored by the female *Anopheles* mosquitoes" [1,2]. "Of over forty recognized species of the *Plasmodium* parasites, five species namely: *Plasmodium vivax, P. ovale, P. malariae, P. falciparum,* and *P. knowlesi* have been reported to infect humans" [3,4,5]. "A few other species such as *P. berghei, P. yoelii, P. chabaudi,* and *P. vinckei,* have also been reported to be infective in other mammals like rodents, and monkeys" [6].

"According to the World Health Organization (WHO), approximately 219 million cases and 440 thousand deaths were reported due to malaria in 2018" [7]. "Nigeria accounts for more malaria cases and deaths than any other country in the world and there were an estimated 68 million malaria cases with over 194,000 deaths in 2021" [8]. "*Plasmodium falciparum,* the most virulent of the malaria parasites, is responsible for the vast majority of the mortality and morbidity associated with malaria infections in Nigeria.Pregnant women and children less than five years are most vulnerable to malaria attack" [3,9].

The widespread resistance of malaria parasites against many antimalarial drugs is a factor in the economic constraints of malaria control. There is evidence of resistance against most antimalarial drugs which is worsened by the emergence of chloroquine-resistant strains of *P. falciparum,* the malaria parasite responsible for most of the death cases every year. This led to the use of artemisinin combination therapy (ACT) and

further search for new phytotherapeutic agents" [10,11].

If not promptly treated with effective medicines, malaria can cause severe illness that is often fatal [3]. Hence, treatment of malaria with combination drug regimens have become the practice of choice because of their increased therapeutic efficacy over monotherapy and the other benefits including decreased cytotoxicity, delay or prevention of the development of drug resistance [12].

But affordability and accessibility limit the use of artemisinin combination therapy and these can lead to poor treatment practices, production of substandard forms of the drug, inadequate patient adherence to prescribed antimalarial regimen, which may in turn lead to treatment failures. Following the problems associated with the implementation of ACT, majority of the populations depend on traditional medical remedies [13], mainly from plants. The traditional herbal medicines are often more available, affordable, and sometimes perceived as being more effective than conventional anti-malarial drugs including artemisinin combination therapy (ACT) [14].

Traditional herbs have been used to treat malaria for thousands of years. Most of these plants are used in the form of monotherapies and only a few plants are taken in combined therapies [15]. Antimalarial plants used in combination may promote the effectiveness of each plant, with efficacy being achieved by the use of a lower dose of each plant extract. This would profer pharmacological benefits, as one extract of the plants clears infection from one body system, and the other clears it from a different site of the same body [14]. Also, synergism with antimalarial agents could be utilized to prevent or delay the emergence *in vivo* of resistant populations of the parasite [16].

Azadirachta indica (Neem) plants from the Meliaceae family are extensively used as traditional medicinal remedies against malaria in the tropics [17,18,19]. Several studies demonstrated that *A. indica* leaf, seed and stem bark extract possess *in vivo* inhibitory activity on *P. falciparum* a sexual stages [18,11,20]. Antimalarial activity of *Ocinum gratissimum* (African basil/clove basil) has also been demonstrated [12]. Although these plants are used in the traditional treatment of malaria, there is the need to scientifically assess the efficacy of the individual plant extracts and their combinations in the treatment of malaria.

The main aim of the present study was to compare the antimalarial effects of *Azadirachta indica* and *Ocimum gratissimum* extracts with chloroquine in *Plasmodium berghei*-infected mice. The specific objectives were to assess the effects of the ethanolic leaf extracts of *A. indica, O. gratissimum*, and their combined extracts in reducing parasitaemia in the albino mice; and the synergistic or inhibitory effects of the combined extracts in treating rodent malaria.

2. MATERIALS AND METHODS

2.1 Study Area

This study was undertaken in Owerri at the Federal Medical Centre Laboratory. Owerri is the capital city of Imo State of Nigeria, located between latitudes 5^0 31^1 and 6^0 27^1 N and Longitudes 7^0 00^1 and 7^005^1, and approximately 100 square kilometers (40sq miles) in area, and consists of three Local Government Areas including Owerri Municipal, Owerri North, and Owerri West (Fig. 1). Owerri has an estimated population of 1,401,873 according to the 2006 census. It is bordered by the Otamiri River to the East and the Nworie River to the South [21].

Owerri has an annual temperature of 20^0C-30^0C, relative humidity of 71%, rainfall distribution of 113.5m. Tropical rainforest is the predominant vegetation in Owerri although its density has drastically reduced due to anthropogenic activities such as urbanization, deforestation and agricultural activities.

2.2 Study Design

The work was an experimental study aimed at investigating the in vivo antimalarial effects of ethanolic leaf extracts of *A. indica* and *O. gratissimum* in *Plasmodium berghei*-infected Swiss albino mice (*Mus musculus* L: Muridae), using chloroquine as the standard drug. The parasites and the mice were sourced from the National Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria. The plant materials (*A. indica* and *O. gratissimum*) were collected from the Botanical Garden of the Ministry of Agriculture and Natural Resources,Owerri, Imo State, Nigeria. Assessment of parasitaemia, and antimalarial effects of the plant extracts were done using standard laboratory analytical procedures at the Federal Medical Centre, Owerri.

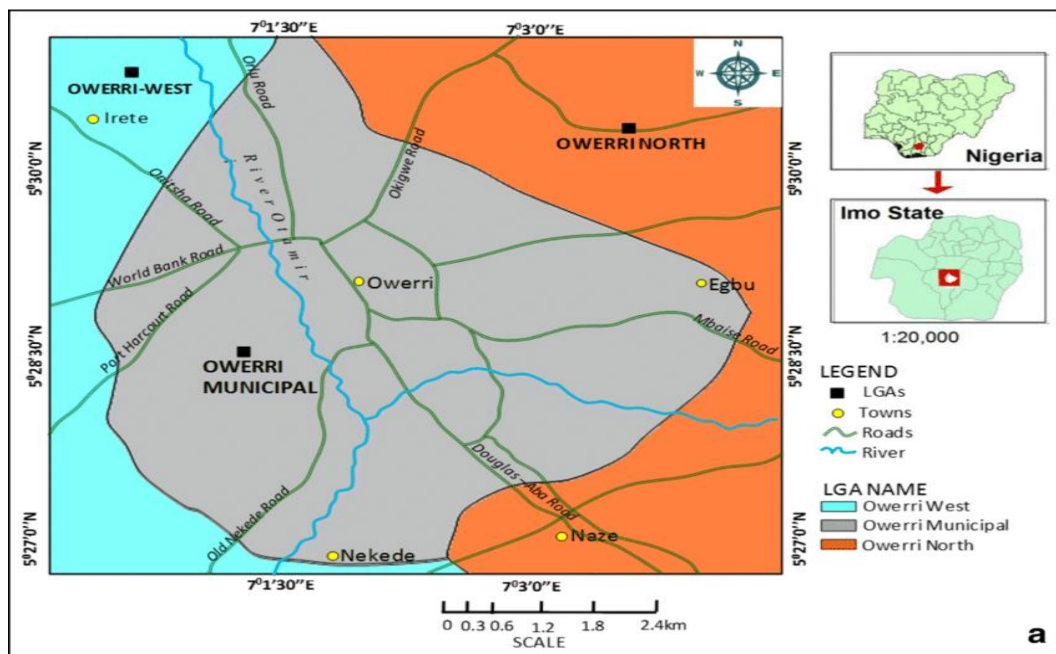

Fig. 1. Showing the Map of Owerri

2.3 Preparation of Plant Materials

Two kilogrammes (2kg) of each plant material was harvested, washed twice in clean water, and allowed to drip dry. The plants leaves were air-dried separately in the laboratory at room temperature for three weeks to a constant weight before pulverizing. A 100g each, of the pulverized leaves of the plants was measured and soaked in 500ml of absolute ethanol (BDL 95%) for 24 hours. The mixture was then filtered using Watman 2.0 filter paper. The filtrate was evaporated to dryness in a Rotary evaporator at a temperature of 40°C. The extracts were kept in a tightly closed bottle in a refrigerator until needed for anti-malaria testing.

The stock solution of each plant filterate was prepared by dissolving 10 g of the extract in 100 ml of distilled water to give a stock concentration of 0.10 g/ml [22].

2.4 Maintenance of Study Animals

One hundred and seventeen mature Swiss albino mice (*Mus musculus*) (25-30g) of either sex free from infection, were used. The animals were housed in mosquito screened-cages lined with wood chip beddings and were stabilized for ten days in the laboratory before being used for the experiments. The mice were maintained on a standard rat diet (Pfizer) and water *ad libitum*. They were kept at ambient room temperature of 12hour light and dark exposure cycles.

2.5 Maintenance of Parasites

Plasmodium berghei (NK65 strain) was used as the rodent malaria parasite in the study. The chloroquine sensitive cyropreserved parasites stored at -80°C were revived, stabilized and maintained by serial passage of blood from infected mice to clean mice that served as donor mice in the study.

2.6 Collection, Preparation of Inoculum, Parasite Inoculation and Establishment of Infection

The blood of a donor mouse was collected in heparinized syringe and diluted in phosphate buffered saline to 10^8 parasitized erythrocytes per ml.

The infection of mice was initiated by needle passage of the *P. berghei* parasite preparation from the donor mouse to healthy test mice via an intraperitoneal route [23]. Parasitaemia was monitored by microscopic examination of Giemsa-stained thin blood smears. The number of parasitised erythrocytes in each of the ten such fields was counted thrice and the average count was computed to give the level of parasitaemia of each mouse [23,24].

The drug chloroquine was administered intramuscularly while the plant extracts and distilled water were administered orally with the aid of an intravenous medicut/mediflon cannula used as an improvised oral cannula [22].

2.7 Acute Toxicity Studies

Albino mice (25-30g) of either sex were used. The median lethal dose (LD_{50}) was determined for each of the neem leaf and clove leaf extracts using the method previously described by Lorke [25]. In the first phase, the mice were divided into three groups with three mice in each group and were administered with the ethanol leaf extracts at doses of 10, 100, and 1000 mg/kg body weight respectively via the oral route. The mice were then observed for signs of toxicity and death for 24 hrs. In the second phase, groups of one mouse each were treated with more specific doses of the extract respectively depending on the result obtained from the first phase and observed for signs of toxicity and death in 24hrs. The final LD_{50} was calculated as the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived.

> LD_{50} = √ Mean of the lowest dose that caused death and the highest dose for which the animal survived.

Matsumura [26] and Corbett et al. [27] classified chemicals based on their LD_{50} values as follows:

Extremely toxic	$LD_{50} \leq 1$ mg/kg
Highly toxic	LD_{50} 1-50 mg/kg
Moderately toxic	LD_{50} 50-500 mg/kg
Slightly toxic	LD_{50} 500-5000 mg/kg
Practically non-toxic	LD_{50} 5000-15000 mg/kg
Harmless	$LD_{50} > 15$ g/kg

Deriving from the outcome of the acute toxicity studies, dosage ranging from 20-60 mg/kg body weight for both extracts of *Azadirachta indica* and *Ocimum gratissimum* leaves were screened for therapeutic activities from which the dose(s) that gave optimum and consistent results were selected. The graded dose used in this study was 50 mg/kg body weight for both extracts.

2.8 Experimental Set up and Treatments

The mice were divided into three experimental groups for the assessment of the efficacy of the plant materials. These were:

Group 1 or Primary Biological Assessment Group
Group 2 or Secondary Biological Assessment Group and
Group 3 or Curative / Therapeutic Group

In Group 1, fifty four mice were used and they were divided into six (6) groups (A,B,C,D,E and F) of nine (9) mice each. The nine (9) mice in each group were sub- grouped into three (1,2,3), of three (3) mice in each subgroup to represent the experimental mice and control mice (i.e positive and negative controls). The mice were infected by method described by Peter and Anatoli [23], and David et al. [28], with 0.2ml of diluted (10^8) parasitised erythrocytes/ml) infected blood each intraperitoneally (i.p), except those in group F that formed control group. Treatment of mice in experimental groups A to E commenced 2 hours after infection at a dose of 10mg/kg body weight for the individual/single plant extracts, and 10 ml/kg for chloroquine and each of combined plant extracts, and treatment was repeated daily with the same dose for 72 hours. Percentage parasitaemia was assessed beginning from 24 hours post treatment, and was repeated on daily basis throughout the treatment period.

Group A - Control (Infected with no treatment).
Group B -Infected and treated with chloroquine (CHQ) (10 ml/kg)
Group C - Infected and treated with *A. indica* leaf extract (10 mg/kg).
Group D - Infected and treated with *O. gratissimum* leaf extract (10 mg/kg).
Group E - Infected and treated with combined extract each of *A. indica* and *O. gratissimum leaf* extracts (10 mg/kg).
Group F - Not infected (Normal Control).

Twenty four hours after the last treatment (96 hours post-infection) blood smears from all the mice were prepared and stained with Giemsa stain for assessment of parasitaemia.

In Group 2, fourty five mice were used and they were divided into fifteen groups of 3 mice per group as follows:

Group A - Infected with no treatment (negative control)
Group B - Infected and treated with 10 ml/kg chloroquine (standard control)
Group C_1 - Infected and treated with 20 mg/kg *A. indica* leaf extract
Group C_2 - Infected and treated with 30 mg/kg *A. indica* leaf extract
Group C_3 - Infected and treated with 40 mg/kg *A. indica* leaf extract
Group C_4 - Infected and treated with 50 mg/kg *A. indica* leaf extract
Group D_1 - Infected and treated with 20 mg/kg *O. gratissimum* leaf extract
Group D_2 - Infected and treated with 30 mg/kg *O. gratissimum* leaf extract

Group D_3 - Infected and treated with 40 mg/kg *O. gratissimum* leaf extract
Group D_4 - Infected and treated with 50 mg/kg *O. gratissimum* leaf extract
Group E_1 - Infected and treated with 15 mg/kg each of *A. indica* and *O. gratissimum* leaf extracts
Group E_2 - Infected and treated with 20 mg/kg each of *A. indica* and *O. gratissimum* leaf extracts
Group E_3 - Infected and treated with 25 mg/kg each of *A. indica* and *O. gratissimum* leaf extracts
Group E_4 - Infected and treated with 30 mg/kg each of *A. indica* and *O. gratissimum* leaf extracts
Group F - Not infected, but administered with 10 ml/kg distilled water (normal control).

Treatment of mice in experimental groups A to E commenced 2 hours after infection at varying doses for the individual/single plant extracts and each of the combined plant extracts, and 10ml/kg for chloroquine . Treatment was repeated daily with the same dose for 72 hours. Twenty four hours after the treatment, blood smears were made from blood bled from the tails of the treated and untreated mice,and stained with Giemsa to assess parasitaemia. Parasite reduction was computed.

In Group 3, eighteen mice were used for the experiment. Mice maintenance, infection, drug preparation and application were same as for the basic 4-day treatment protocol. Treatment commenced on day four when parasitaemia was well observed in the infected mice and percentage parasitaemia was assessed beginning from 24h post treatment. Fifty milligram per kilogram body weight of *A. indica* leaf extract, 50mg/kg body weight of *O. gratissimum* leaf extract, 10ml/kg body weight of chloroquine, and 30mg/kg body weight each of *O. gratissimum* leaf extract and A. indica leaf extract were used for the treatment.

2.9 Assessment of Parasitaemia

Before parasite inoculation and at 24hours post treatment period,the experimental mice were bled from their tails, thin blood smears were made by fixing for 5 minutes using methanol stained with 10% Giema stain in phosphate buffer, P^H 7.2, and examined microscopically under oil immersion at x100. Parasitaemia level was determined by counting the parasites observed in 10 fields of approximately 100 erythrocytes per field. The difference between the mean values of the experimental group was calculated and expressed as percentage parasitaemia inhibition using the following equation:

$$\% \text{ Inhibition} = \frac{\text{Parasitised RBC in Negative Control} - \text{Parasitised RBC in study group} \times 100}{\text{Parasitised RBC in Negative Control}}$$

2.10 Data Analysis

Data was analysed using the statistical package for social sciences (SPSS) version 2.0. Tabulations, percentages and graphs were used to present results. Statistically significant differences were analyzed using Analysis of variance (ANOVA). Graphs were plotted using Microsoft Excel, version 2007.

3. RESULTS

3.1 Acute Toxicity Study and Behavioural Effects of the Plants' Leaf Extracts

Acute toxicity test of the A. indica leaf extract at concentrations of 100 and 1000 mg/kg produced mortality after 24hrs of observation. The median lethal dosage (**LD_{50}**) of this extract was 31.80 mg/kg. However, the animals were observed to experience slow movement within the first six hours of the extracts. The O. gratissimum leaf extract did not show any mortality for the 100 mg/kg dose and it recorded a higher lethal dosage of 1264.9 mg/kg (Table 1). According to Matsumura [26] and Corbett et al. [27], the *A. indica* leaf is highly toxic while the *O. gratissimum* is classified as slightly toxic.

3.2 Intensity of Parasitaemia

Few minutes prior to infection of the mice, no malaria parasite was observed in the peripheral blood of all the experimental animals. Detectable parasitaemia was recorded from day 3 post infection (p.i). On the 4th day of post infection, parasite intensity (Table 2), was between 12030±61.83 and 12292±101.61 in all the infected mice. Parasite intensity was not significantly different in the treatment groups B,C,D and E ($P>0.05$; P= 0.958, 1.00, 0.958, and 0.99).

After treatment of mice with 10mg/kg body weight on the day 4 post infection, the parasite intensity ingroup A(infected but not treated) rose significantly from12174±1.00 to 136891±1.00 ($P<0.05$; P=0.00). The parasite intensity of

miceinfected and treated with chloroquine in (group B, standard control) significantly decreased to 3.00 ± 0.58 ($P<0.05$; $P=0.00$). There were no significant reductions in parasite intensity among those treated with 10 mg/kg body weight of mice with the extracts of *A. indica* and *O. gratissimum* separately or combined.

Table 3 indicates that few minutes before infection of the mice, there were no significant differences in parasite intensity in both the control groups A, B, and F, and treatment groups C_1-C_4,D_1-D_4 and E_1-E_4 ($P<0.05$; $P=0.00$). No parasite was observed in the peripheral blood of all the experimental mice.

Two hours after infection, parasite intensity was between 12316±656.81 and 12705±261.40 in all the infected mice. Parasite intensity was not significantly different in treatment groups B, C_1-C_4,D_1-D_4 and E_1-E_4 ($P>0.05$; $P=1.00$), that were infected with the parasites.

Table 1. Lethal Dose (LD_{50}) of the Ethanol Extracts of *Azadirachta indica* and *Ocimum gratissimum*

Extract	LD_{50} (mg/kg)	Verdict
A. indica	31.62	Highly toxic
O. gratissimum	1264.9	Slightly toxic

Table 2. Changes in parasite intensity before infection, after infection, and after administration of ethanolic leaf extracts of *A. indica,* and *O. gratissimum* in *P. berghei*-infected Mice (Primary Biological Assessment of *A.indica* and *Ogratissimum* Extracts Efficacy)

Experimental groups	Parasite intensity					
	Before infection($x10^3$)		After infection ($x10^3$)		After treatment ($x10^3$)	
A - Infected, No Treatment	0.000± 0.00	0.000 Sig P<0.05	12174.00 ± 1.00	0.000 Sig P<0.05	136891.00 ± 1.00	0.000 Sig P<0.05
B - 10 ml/kg of Chloroquine	0.000± 0.00	0.000 Sig P<0.05	12030.00 ± 61.83	0.958 NS P>0.05	3.00 ± 0.58	0.000 Sig P<0.05
C - 10 mg/kg of *A. indica*	0.000± 0.00	0.000 Sig P<0.05	12267.00 ± 274.64	0.754 NS P>0.05	3601.00 ± 330.77	0.157 NS P>0.05
D - 10 mg/kg of *O. gratissmum*	0.000± 0.00	0.000 Sig P<0.05	12292.00± 101.61	1.000 NS P>0.05	4367.00 ± 275.99	0.992 NS P>0.05
E - 10 mg/kg each of *A.i & O.g*	0.000± 0.00	0.000 Sig P<0.05	12224.00 ± 22.61	0.999 NS P>0.05	1710.00± 438.25	0.416 NS P>0.05
F-Not Infected, Normal Control	0.000± 0.00	0.000 Sig P<0.05	0000.00 ± 0.00	0.000 Sig P<0.05	0.00 ± 0.00	0.000 Sig P<0.05

Key: (A.i)=Azadirachta indica, (O.g)=Ocimum gratissimum

Table 3. Changes in Parasite Intensity before Infection, after Infection, and after Administration of Ethanolic Leaf Extracts of *A. indica* and *O. gratissimum* in *P. berghei*-infected Mice at different Concentrations (Secondary Biological Assessment of *A. indica* and *O. gratissimum* Extracts Efficacy)

Experimental groups	Parasite intensity					
	Before infection($x10^3$)		After infection($x10^3$)		After treatment ($x10^3$)	
A - Infected, No Treatment	0.000± 0.00	0.000Sig P<0.05	12467.00 ± 611.01	1.000 NS P>0.05	312526.00 ± 149404.59	0.000 Sig P<0.05
B - 10 ml/kg of chloroquine	0.000± 0.00	0.000 Sig P<0.05	12316.00 ± 656.81	1.000 NS P>0.05	3.00 ± 0.58	1.00. NS P>0.05
C_1 – 20 mg/kg of *A. indica*	0.000± 0.00	0.000 Sig P<0.05	12439.00 ± 356.31	1.000 NS P>0.05	3929.00 ± 53.15	1.00. NS P>0.05
C_2 – 30 mg/kg of *A. indica*	0.000± 0.00	0.000 Sig P<0.05	12504.00 ± 517.04	1.000 NS P>0.05	3614.00 ± 16.70	1.00. NS P>0.05
C_3 – 40 mg/kg of *A. indica*	0.000± 0.00	0.000 Sig P<0.05	12450.00 ± 671.38	1.000 NS P>0.05	3372.00 ±25.51	1.00. NS P>0.05
C_4 – 50 mg/kg of *A. indica*	0.000± 0.00	0.000 Sig P<0.05	12363.00 ± 469.67	1.000 NS P>0.05	3115.00 ± 13.01	1.00. NS P>0.05
D_1 – 20 mg/kg of *A. indica*	0.000± 0.00	0.000 Sig P<0.05	12472.00 ± 408.24	1.000 NS P>0.05	4872.00 ± 37.02	1.00. NS P>0.05
D_2- 30 mg/kg of *O. gratissimum*	0.000± 0.00	0.000 Sig P<0.05	12658.00 ± 415.44	1.000 NS P>0.05	4646.00 ± 45.13	1.00. NS P>0.05
D_3- 40 mg/kg of *O. gratissimum*	0.000± 0.00	0.000 Sig P<0.05	12508.00 ± 370.66	1.000 NS P>0.05	4422.00 ± 10.50	1.00. NS P>0.05
D_4- 50 mg/kg of *O. gratissimum*	0.000± 0.00	0.000 Sig P<0.05	12625.00 ± 341.15	1.000 NS P>0.05	4129.00 ± 91.35	1.00. NS P>0.05
E_1- 15 mg/kg each of *A.i* & *O.g.*	0.000± 0.00	0.000 Sig P<0.05	12551.00 ± 155.38	1.000 NS P>0.05	2785.00 ± 20.59	1.00. NS P>0.05
E_2- 20 mg/kg each of *A.i* & *O.g.*	0.000± 0.00	0.000 Sig P<0.05	12705.00 ± 261.40	1.000 NS P>0.05	2576.00 12.50	1.00. NS P>0.05
E_3-25mg/kg each of *A.i.* & *O.g.*	0.000± 0.00	0.000 Sig P<0.05	12578.00 ± 241.33	1.000 NS P>0.05	2321.00 ± 16.56	1.00. NS P>0.05
E_4- 30 mg/kg each of*A.i.* & *O.g.*	0.000± 0.00	0.000 Sig P<0.05	12396.00 ± 221.74	1.000 NS P>0.05	2106.00 ± 31.76	1.00. NS P>0.05
F-Not Infected,Normal Control	0.000± 0.00	0.000 Sig P<0.05	0.000 Sig P<0.05	0.000 Sig P<0.05	0000.00± 0.000	0.00 Sig P<0.05

Ninety-six hours after treatment with varying doses (10 ml/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 40 mg/kg, and 50 mg/kg) body weight of the mice, parasite intensity in group A (infected but not treated) rose significantly from 12467±611.01 to 312526 (P>0.05; P=1.00). The parasite intensity in group B (infected and treated with chloroquine) significantly decreased to 3.00±0.58 (P>0.05; P=1.00). There were non-significant reductions in parasite intensity among those treated with the plant extracts of *A. indica* and *O. gratissimum* and their combined regimens (P>0.05; P=1.00).

Table 4 shows that few minutes prior to infection, there were no significant differences in parasite intensity in both the control groups A, B, and F, and treatment groups C, D, and E, (P<0.05; P=0.00). No parasite was observed in the peripheral blood of all the experimental mice.

Twenty-four hours after infection, parasite intensity was between 136853±1022.01 and 138633±852.31 in all the infected mice. Parasite intensity was not significantly different in treatment groups B,C,D, and E (P>0.05; P=0.995, 0.599, 0.9902, and 1.00), that were infected with the parasites.

Twenty-four hours after last treatment with 30mg/kg and 50mg/kg body weight of the mice, parasite intensity in groups A (infected but not treated) rose significantly from 138083±1.00 to 181358.00±1.00 (P<0.05; P=0.00). The parasite intensity in groups B (infected and treated with chloroquine) significantly decreased to 3.00±0.58 (P<0.05; P=0.00). There were non-significant reductions in parasite intensity among those treated with plant extracts of *A. indica,*and *O. gratissimum* and their combined regimens (P>0.05; P=1.00).

3.3 Antimalarial Effects of Different Dosages of the Plants Leaf Extracts

The treatment of animals with different dosages of ethanolic leaf extracts of the plants showed a different response to increased dosage in line with protection against infection (Fig. 2). The parasitaemia in the infected but untreated group increased with days of infection while the parasite-infected and treated groups had significant reduction (P<0.05; P=0.00, 0.009, 0.002, and 0.00) in parasitaemia level from day one to seven. From day 1 to 7 of the treatment period, all groups showed significant difference in parasitaemia levels as compared to the untreated control group (P>0.05; P=0.122, 0.060, and 0.088).

The extract of *A. indica* at 20 mg/kg, 30 mg/kg, and 40mg/kg showed parasitaemia reduction of 57.80%, 59.0%, and 60.10%; and 62.50%, 63.60%, and 64.50% on days 4 and 7 respectively, while the 50 mg/kg of *A. indica* treated group recorded 61.40% parasitaemia reduction on day 4 and 66.00% on day 7. Values observed with 20 mg/kg, 30 mg/kg and 40 mg/kg of *O.gratissimum* were 49.08%, 52.70%, and 55.10%; and 54.10%, 57.00%, and 59.88% on days 4 and 7, respectively, while 50 mg/kg of *O. gratissimum* gave 56.88% and 61.10% reduction on days 4 and 7,respectively (Fig. 4). Combination doses of 15 mg/kg, 20 mg/kg, 25 mg/kg and 30 mg/kg of each of the plant leaf extracts gave a significantly higher reduction in parasitaemia as compared to combined treatments with 40 and 50mg/kg. Maximum reduction of 78.65% parasitaemia was attained for the extracts combinations of 30mg/kg of each plant extract on the seventh day, indicating synergistic action of the extracts. By the twenty-eight day post treatment, the parasitaemia had gone below detectable levels in all treated groups. The plant extracts showed increase in parasitaemia inhibition with increase in dose administered on the infected animals, i.e dose-dependent inhibition of parasitaemia, and even at the higher dosages, the animals survived longer than the chloroquine treated ones.

The therapeutic effect of the leaf extracts of the plants on the infected animals (Fig. 3) showed that the animals responded to the extracts within the first seven days of treatment with 69.89% reduction of parasitaemia for *O. gratissimum* treated group, 71.11% for *A. indica* treated group, and 82.70% for combined extracts of *A. indica* and *O. gratissimum* treated groups before the parasitized red blood cells started to increase which later led to the deterioration of the health condition of the animals. The antimalarial action of the combined leaf extracts of *A. indica* and *O. gratissimum* (82.70%) was close to that of chloroquine (97.98%).

4. DISCUSSION

The *in vivo* antimalarial activities of *A. indica* and *O. gratissimum* leaf extracts were studied in *P. berghei*-infected mice to determine the treatment outcomes of the extracts from January to March, 2021.

Table 4. Changes in Parasite Intensity before Infection, after Infection, and after Administration of Ethanolic Leaf Extracts of *A. indica*, and *O. gratissimum* in *P. berghei*-infected Mice (Therapeutic Effects of *A. indica and O. gratissimum*)

Experimental groups	Parasite intensity					
	Before infection($x10^3$)		After infection ($x10^3$)		After treatment ($x10^3$)	
A - Infected, No Treatment	0.000 ± 0.00	0.000 Sig P<0.00	138083.00 ± 1.000	0.000 Sig P<0.05	181358.00 ± 1.00	0.000 Sig P<0.00
B – 10 ml/kg of Chloroquine	0.000 ± 0.00	0.000 Sig P<0.00	138633.00 ± 852.31	0.995 NS P> 0.05	3.00 ± 0.58	0.000 Sig P<0.05
C - 50/mg/kg *A. indica*	0.000 ± 0.00	0.000 Sig P<0.00	136853.00 ± 1022.01	0.599 NS P> 0.05	1957.00 ± 694.54	0.137 NS P>0.05
D -50 mg/kg of *O. gratissimum*	0.000 ± 0.00	0.000 Sig P<0.00	137971.00 ± 903.51	0.9902NSP> 0.05	3083.00 ± 93.54	0.625 NS P>0.05
E – 30 mg/kg each of *A.i* & *O.g*	0.000 ± 0.00	0.000 Sig P<0.00	137634.00 ± 2301.11	1.000 NS P> 0.05	1235.00 ± 111.53	0.172 NS P>0.05
F-Not Infected,Normal Control	0.000 ± 0.00	0.000 Sig P<0.00	0.00± 0.00	0.000 Sig P< 0.05	0.00 ± 0.00	0.000 Sig P<0.05

Key: (A.i)=Azadirachta indica, (O.g)=Ocimum gratissimum

Before parasite inoculation and at 24 hours post treatment period, parasitaemia was monitored by microscopic examination of Giemsa-stained thin blood smears in all the experimental mice. The experimental mice were placed into various groups for the assessment of the efficacy of the plant materials used in the study. The significant increase in the level of parasitaemia in the infected untreated group recorded from day 1 to day 7, as symptomised by the enlargement of the red blood cells, anaemia, loss of appetite, and loss of weight as major clinical signs observed during the course of treatment is in tune with the view that parasitaemia increases progressively after infection until the point of death if no suitable treatment is administered [29,9]. There were significant increases in parasitaemia reduction with increase in doses of the extracts administered in the treated groups. This indicates that as the dose increases, the strength of the antiplasmodial activity of the plant extracts also increases.

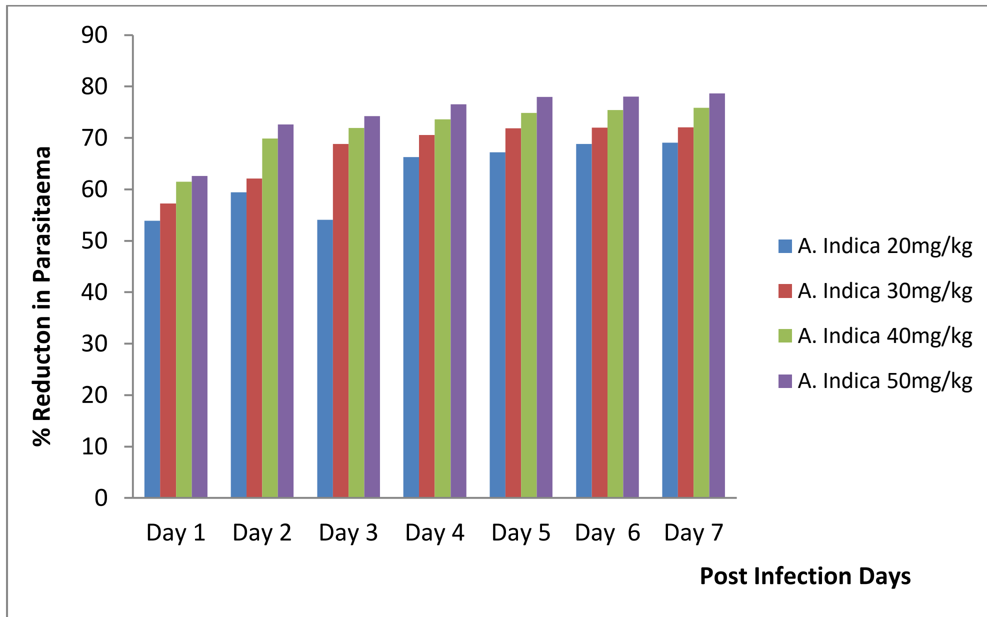

Fig. 2a. Effects of different doses of *A. indica* Extract on Parasitized RBC of *P. berghei* infected mice

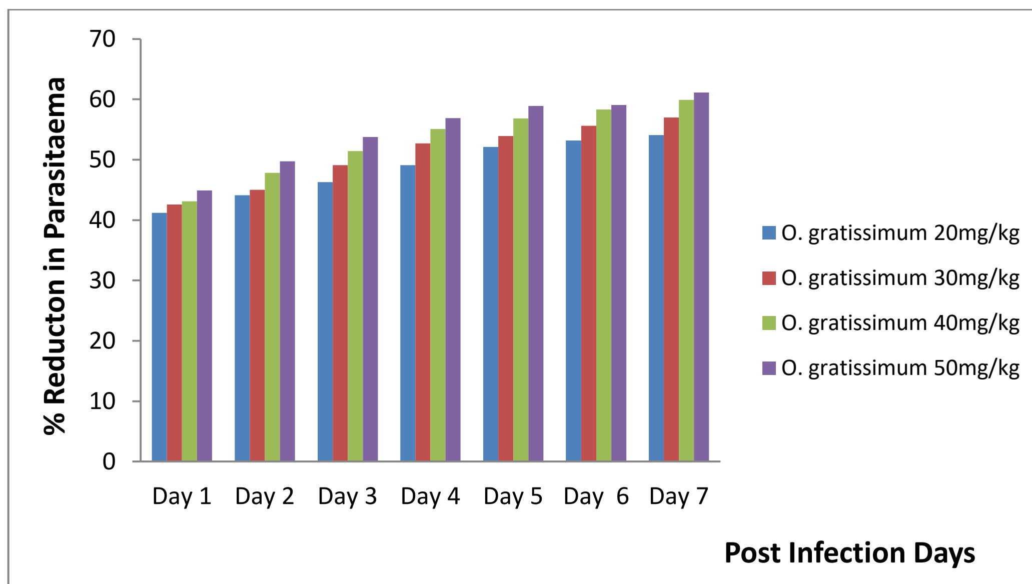

Fig. 2b. Effects of different doses of *O. gratissimum* Extracts on Parasitized RBC of *P. berghei* infected mice

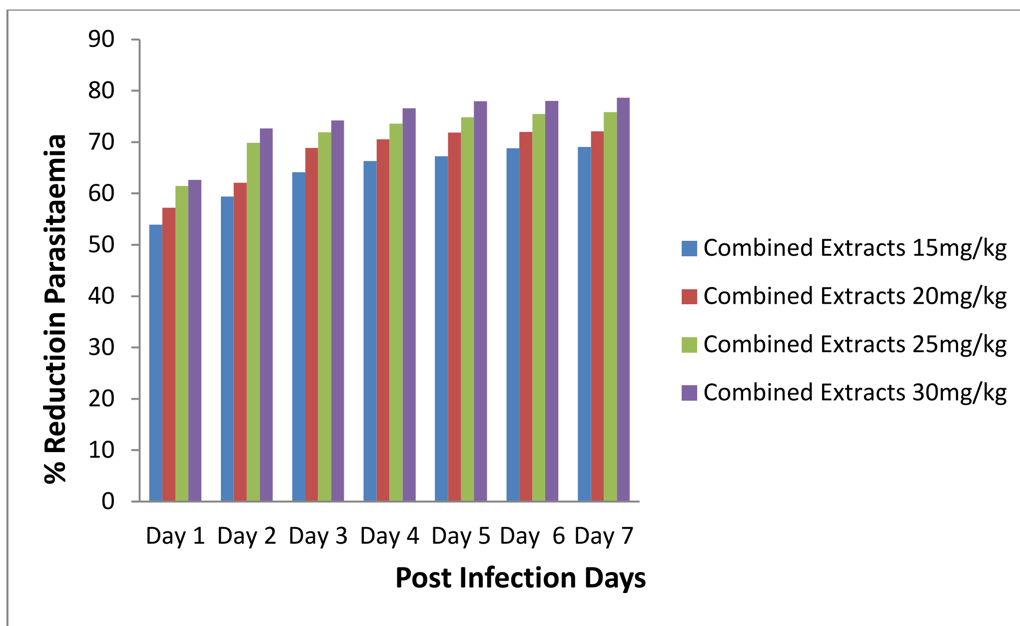

Fig. 2c. Effects of different doses of combined extracts of *A. indica* and O. *gratissimum* on parasitized RBC of *P. berghei* infected mice

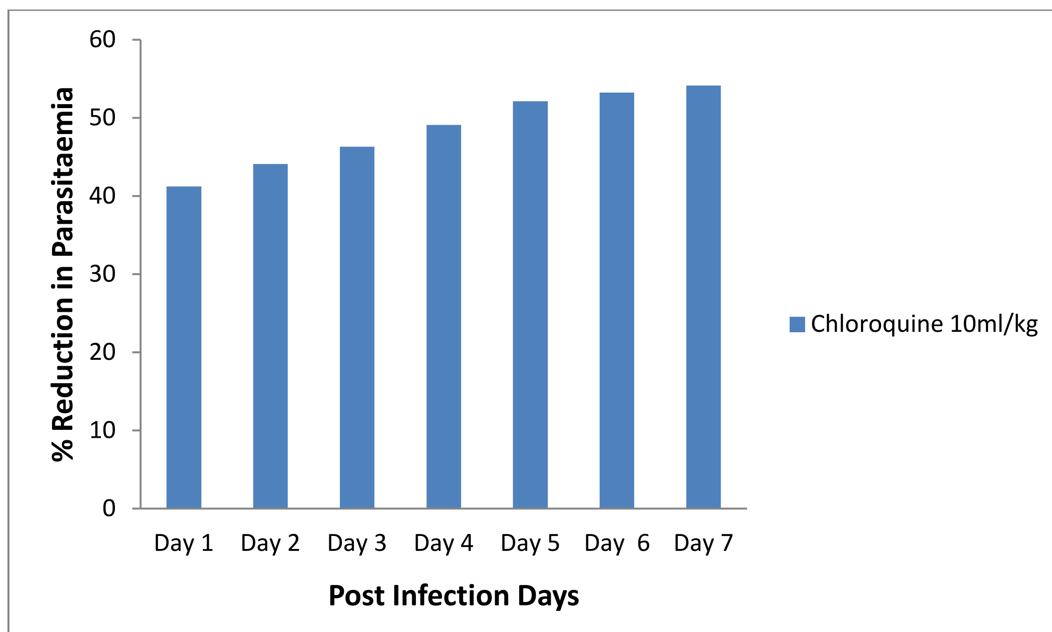

Fig. 2d. Effects of chloroquine on parasitized RBC of *P. berghei* infected mice

The reduction in parasitaemia was highest in the group administered with the combined extracts of *A. indica* and *O. gratissimum* (Fig. 3, [82.70%]) than those administered with single plant extracts. This may be attributed to the antiplasmodial activities of the various ethanolic extracts, on one hand, and result of the synergistic effect of the compounds or their metabolites on the other hand. These findings agree with the reports of Alehegn et al. [30], Ceravolo et al. [31], Maximus et al. [32], Oladeji et al. [33], Singh et al. [34], Ekpo and Ekanemesang [35], and Cissy et al. [36], which indicate that phytochemicals are responsible for the antimalarial activities of many plants. Also, these metabolites may be acting individually or in synergy with one another to produce antimalarial activity. This is attributable to

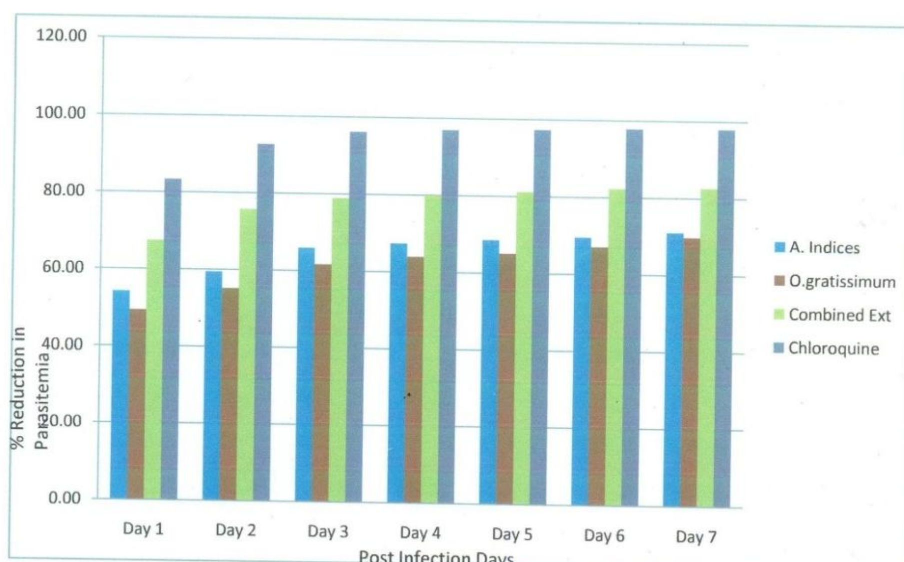

Fig. 3. Therapeutic effect of ethanolic extracts of the plants leaves on albino mice infected with *P. berghei*

the ability of the plant extracts to reduce the intensity of parasitaemia in the infected and treated groups, which is in tune with studies reported by Chaniad et al. [37], Joseph & Samson [38], and Osonwa et al. [10]. The activity of the combined leaf extracts of A. indica and O. gratissimum was also observed to be better than the individual/separate extracts of A. indica and O. gratissimum, which corroborates the results observed in their antiplasmodial activity [39,10,40].

5. CONCLUSION

From observations, the individual leaf extracts of *A. indica* and *O. gratissimum* have been shown to be effective in the treatment of malaria parasite in mice by reducing the parasite intensity.All the therapeutic observations were more pronounced in the combined extracts treatments indicating synergistic action of the extracts. It is therefore recommended that the coadministration of these locally used plants, especially at the concentrations investigated in this study be taken for the treatment and management of malaria fever as their action is very close to that of the antimalarial drug, chloroquine which was the standard drug used in this study.

ETHICAL APPROVAL

Approval for the study was obtained from the Ethical Committee, Public Health Unit of the Owerri Municipal Council(OWMC), Owerri, Imo State, Nigeria. The caring and experimental use of the mice was in accordance with the National Institute of Health Guidelines for care of Laboratory Animals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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