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Full Length Research Paper

Antiplasmodial Effect of Ethanolic Leaf Extract of *Irvingia gabonensis* on *Plasmodium berghei* in Mice.

Malann, Y.D., Aminu, K. and Olanrewaju, C.A.
Department of Biological Sciences, Faculty of Science, University of Abuja, Abuja, F.C.T

**Corresponding author: Malann, Yoila David*

Abstract
The ethanolic leaf extract of Irvingia gabonensis was investigated for antimalarial activity against Plasmodium berghei infected mice. The lethal dose was determined to ascertain the safety of the extract in mice to be 1000mg/kg body weight. Ethanolic leaf extract of I. gabonensis (100, 250, 500 mg/kg) was administered orally to Plasmodium berghei infected mice. The crude leaf extract of I. gabonensis significantly (P≥0.05) reduced the parasitaemia count in the erythrocytes of P. berghei infected albino mice and significantly (P≥0.05) increases the percentage of parasite inhibition. It was also found that higher doses of the extract (500mg/kg) exhibited higher antiplasmodial activities than lower doses (100mg/kg and 250mg/kg). So also, antiplasmodial activity of the doses increases as the duration of the experiment progresses. These results show that the ethanolic leaf extract of I. gabonensis possesses significant antiplasmodial activity thus rationalizing its traditional use in malaria therapy.

Keywords: *Antiplasmodial, Irvingia gabonensis, Plasmodium berghei, Mice, Parasitaemia.*

Introduction

Malaria is mosquito-borne infectious disease of human and other animals caused by eukaryotic protists of the genus *Plasmodium* (Ali *et al*., 2004). The disease results from the multiplication of *Plasmodium* parasites within red blood cells, causing symptoms that typically includes fever and headache, in severe cases progressing to coma or death (Betemariam and Yayeh, 2002). It is widespread in tropical and sub-tropical regions, including much of sub-Saharan Africa, Asia and the Americas.

There were an estimated 225 million cases of malaria worldwide in 2009 (WHO, 2010). An estimated 655, 000 people died from malaria, accounting for 2.23% of deaths recorded worldwide (Betemariam and Yayeh, 2002). Ninety percent of malaria-related deaths occur in sub-Saharan Africa, with the majority of deaths being young children. In Nigeria, the disease is a major health problem with stable transmission throughout the country (Bloland, 2001). It accounts for about 50% of out-patient consultation, 15% of hospital admission and is the prime amongst the top three causes of death in the country (Muregi *et al*., 2007). More importantly, it is a social and economic problem which consumes about 5 million US Dollar in various control attempts (Hilou *et al*., 2006). In addition to its direct health impact, malaria imposes a huge economic burden on afflicted individuals and nations, through high health care cost, missed days at work or school, and reduces economic output and productivity. Currently, multi-drug resistance has become one of the most important problems impeding malaria control efforts (Checchi *et al*., 2006).

According to Betemariam and Yayeh (2002), malaria transmission can be reduced by preventing mosquito bites by distribution of mosquito nets and insect repellents, or by mosquito-control measures such as spraying insecticides and draining standing water where mosquitoes breed. The challenge of producing a widely available vaccine that provides a high level of protection for a sustained period is still to be met, although several are under development (Checchi *et al*., 2006).

A number of medications are also available to prevent malaria in travelers to malaria-endemic countries (Hilou *et al*., 2006). The acquisition of multidrug resistance resulting to reduction in the effectiveness of a drug in curing a disease or improving a patient's symptom has led to a serious impediment to improved health care issues. The diversity of resistance types will require that public health measures to control malaria should be region specific (Snow *et al*., 2005).

Plasmodium berghei is a species of malarial *Plasmodium* that infects African thicket rats and is passed by the mosquito *Anopheles dureni* (Simonsen *et al*., 2001). Scientists commonly use it to model human hepatic malaria because it is transmissible to laboratory rats using Anopheles mosquito, which can be maintained in laboratory conditions. Malaria plasmodia are highly similar in their patterns of infection and life cycle, making the *berghei* strain, which is not transmittable to humans, a safe choice for experimentation and observation (Snow *et al*., 2005).

The attempts to discover other antimalarial agents, mainly from plant sources may provide antimalarial drugs directly as in the case of quinine from cinchona bark or they may supply template molecules on which to base further new structure by organic synthesis from

Artemisia annua (Belding, 1942; Olliaro and Trigg, 1995). In Africa, up to 80 percent of the populations still rely on herbal medicine to treat malaria and other diseases, because of their affordability and accessibility.

Plants have constituted the nucleus of drug development for a very long time. Wild and cultivated plants have provided humans with cures for thousands of years. These medicines are safe and environment friendly. Most drugs have been derived from plants which include the anti-malarial drug artemisinin, anticancer drugs and antibacterial drugs. Bioactive natural products often occur as part of related molecules so that it is possible to isolate a number of homologues and obtain structured activity information. It shows that compounds found from screening of natural products can be utilized by traditional medicinal chemistry (Snow *et al*., 2005). During the last few decades there has been an increasing interest in the study of medicinal plants and their traditional use in different parts of the world. Plants have always been considered to be a possible alternative and rich source of new drugs.

Irvingia gabonensis family Irvingiaceae locally known as ***ogbono*** in Nigeria and commonly called 'African mango' or "wild mango", is an indigenous forest tree belonging to the group of plants classified as "Non Timber Forest Products (NTEP), the natural habitat of *I. gabonensis* extends from Senegal to Sudan and South of Angola. In some Nigerian tribes like Nupe it is called "pekpeara"; "Ogwi" in bini; "Ogbono/ugiri depending on the variety in Igbo; "uyo" in Efik and "oro" (tree)"apon" (kernel) in Yoruba. It is an economic tree cultivated mainly for its seeds. The leaves and stem bark are employed in the traditional African medicine against fever and stomach upset. The seeds are popularly used as soup thickener and are responsible for the characteristic appetizing flavour of the Nigeria *Ogbono* soup (Onyeike *et al.,* 1995)

Materials and Methods

Study Area

This study was carried out at Biotechnology Laboratory, Department of Chemistry, Sheda Science and Technology Complex (SHESTCO), FCT, Abuja.

Collection of the Plant Material

Fresh leaves of *I. gabonensis* were collected freshly in the wild, at Ake village, Edo State from its tree and were authenticated by professor Olorode of Department of Biological Sciences, University of Abuja before transferring them to the laboratory for analysis.

Preparation of Plant Extract

The methods described by Shakya, (2012) were employed in preparation of the plant extract. The leaves were cleaned, air-dried at room temperature for 30 days and crushed into coarse power using pestle and mortar. The 225gram of the powdered material was macerated with 350ml of 80% ethanol for 48hour duration. It was further filtered and the filtrate was concentrated in a hot plate oven below 40°C to get dryness. The ethanol extract was used in the all studies with doses expressed in mg / kg body weight of the animal.

Phytochemical Screening

For the determination of the phytochemical compositions of the plant extract of *I. gabonensis*, 25g of ground sample was dissolved in 100ml of distilled water in a conical flask. The mixture was covered and allowed to stand for 3 hours with occasional stirring. It was filtered with a Whatman no. 2 filter paper and the filtrate was stored in plastic container and kept in ambient temperature prior to analysis. The test was carried out using the method of Trease and Evans (1989) for determining the presence of carbohydrate, glycosides, tannins, flavonoids, alkaloids, saponins and phlobotanin.

Study Design

A complete experimental block design was employed in this study. A total of 30 albino mice were randomly assigned into three treatment group and two control group (negative and positive control) with six albino Swiss mice in each group. All animal experiments were conducted in Sheda Science and Technology Complex, SHESTCO, Department of Chemistry, Biotechnology Laboratory, FCT, Abuja.

Experimental Animals and Feeding

Thirty male albino Swiss mice weighing (20-25g) were obtained from National Veterinary Research Institute, Vom, Plateau State, Nigeria. They were acclimatized for 14 days in metal cages in a room maintained at temperatures between 27-30^{0}C with 12hour light dark cycle. They were fed *ad libidum* with growers mash. All the mice were allowed free access to food and clean water throughout the experimental period. Good hygiene was maintained by constant cleaning and removal of feces and spilled feed from cages daily.

Acute Toxicity Study (LD_{50}) of the Plant Extract

The oral acute toxicity of the ethanol extract was estimated in albino mice of weight between 20-25g by medium lethal dose (LD_{50}) (Lorke, 1983). Three groups of three albino Swiss mice each were used and the animals were given extracts at doses of 100, 200, 1000 mg/kg body weight respectively. Extracts used were dissolved in distilled water before administration. The animals were monitored for 24hours and number of deaths per group recorded. The albino mice were observed for gross behavioural changes such as feeding, hair erection, shivering, heat-seeking behaviour, mortality and other signs of toxicity manifestation. The albino Swiss mice were allowed free access to food and clean water during the experiment.

In Vivo Antimalarial Activity of Crude Extract

Methods described by Shakya, (2012) were employed in determining the *in vivo* antimalarial activity of the crude leaf extract of *I. gabonensis.*

Parasite Inoculation

A strain of *Plasmodium berghei* Chloroquine-sensitive was obtained from National Veterinary Research Institute, Vom, Plateau State, Nigeria. The Albino mice previously infected with *P. berghei* having variable parasitaemia were used as donors. Parasitize erythrocytes were obtained from a donor infected mouse by cardiac puncture with a sterile needle and blood was collected in a petri dish with the help of anticoagulant (heparin). The blood was then diluted with normal saline (0.9%) based on the parasitaemia of the donor albino Swiss mice in such a way that 1 ml blood contain $5x10^7$ infected erythrocytes. Each mouse received 0.2 ml diluted blood containing $1x10^7$ *P. berghei* infected erythrocytes by intra-peritoneal route. To avoid variability in parasitaemia, the blood collected from all donor albino mice was pooled together, and the parasite was subsequently maintained by serial blood passage from mouse to mouse every 5 – 7 days.

Administration of the Extract

Twenty five mice were used after three days of infection with *P. berghei*, then divided into five groups-A, B, C, D and E. Groups A, B and C were given daily doses of 100, 250 and 500 mg/kg each of leaf extract of *I. gabonensis* respectively for four consecutive days. Group D animals were treated daily with 5mg/kg dose of chloroquine while Group E animals were treated daily with 1ml of normal saline injected intraperitonally. Treatment started on day 3 after infection (Mukherjee, 2002; Saidu *et al*., 2000; Adzu *et al*., 2007).

Laboratory Examination of the Blood Samples

The blood samples were examined for four days after the third day of infection. A modified peters 4-day suppressive test against *P. berghei* infected Swiss albino mice was employed. On the fifth day (day 4 after treatments) blood was collected from the tail of each mouse. Thin film microscopic examination of the blood samples was conducted. A drop of the blood was placed on one end of the slide. Using a spreader slide, the blood was dispersed over the slide length by gently pushing the spreader slide across the blood backwards. The thin blood film was air dried for about 5 minutes. The blood was fixed by immersing the slide into methanol for 1 second and then Giemsa stain was used to stain the slides for 3 minutes. The slides were later washed with distilled water and air dried. An oil immersion was added to the slide to aid clearer view of the parasite under x100 objectives.

The parasitaemia was determined by counting minimum of three fields per slide with 100 RBC per field. The percentage suppression of parasitaemia was calculated for each test concentration by comparing the parasitaemia in infected mice treated groups with the controls.

Percentage inhibition of the parasite was calculated by the following formula.

$$\%PI = \frac{\text{Parasitaemia in negative control} - \text{Parasitaemia in study group}}{\text{Parasitaemia in negative control}} \times 100$$

The above formula was used for all calculations from day 1 to day 4.

Data Analysis

The results were expressed as mean ± standard deviation. The data was analyzed using student t-test and differences between the means were considered significant at ($P< 0.05$).

Results

Preliminary Qualitative Phytochemical Screening

Table 1 shows the preliminary qualitative phytochemical compositions of the aqueous leaf extract of *Irvingia gabonensis*. The result obtained showed that the aqueous leaf extract of *Irvingia gabonensis* contain alkaloids, tannins, glycosides, flavonoids, steroids, saponins, steroids, resins and carbohydrates.

Acute Toxicity Tests

The median lethal dose LD_{50} of the ethanolic leaf extract was estimated to be approximately ≥1000mg/kg having shown no mortality rate from 100 to 1000 doses tested. The extract was assumed to be safe. Invariably, the experimental doses used (100mg/kg, 250mg/kg and 500mg/kg) were relatively safe.

Table 1: Phytochemical Composition of the Aqueous Leaf Extract of *I. gabonensis*

Compounds	Results
Alkaloids	++
Tannins	+
Glycosides	+
Saponins	+
Flavonoids	+++
Steroids	+
Phlobotanins	-
Resins	+
Carbohydrate	+++

Key:

+++	=	*Intensely present*
++	=	*Moderately present*
+	=	*Minutely present*
-	=	*Absent*

Antiplasmodial Activity

Table 2 shows the antiplasmodial activity (parasitaemia count) of the leaf extract of *I. gabonensis*. On treatment with 100mg/kg of the leaf extract of *I. gabonensis*, the parasitaemia count ranged from 16±1.2 as at the fourth day of post infection to 12±1.8 as at the 7th day after infection. On treatment with 250mg/kg of the leaf extract of *I. gabonensis*, the parasitaemia count ranged from 14±3.5 as at the fourth day of post infection to 10±2.1 as at the 7th day after infection. On treatment with 500mg/kg of the leaf extract of *I. gabonensis*, the parasitaemia count ranged from 14±3.1 as at the fourth day of post infection to 7±1.3 as at the 7th day after infection. On treatment with 5 mg of chloroquine (Positive Control) of the leaf extract of *I. gabonensis*, the parasitaemia count ranged from 12±1.8 as at the fourth day of post infection to 5±1.1 as at the 7th day after infection. For the negative control (Saline water), the parasitaemia count ranged from 19±3.3 as at the fourth day of post infection to 28±2.5 as at the 7th day after infection.

Table 2: Antiplasmodial Activity (Parasitaemia count) of *I. gabonensis* in the Erythrocytes of the Swiss Albino mice

PARASITAEMIA COUNT

Day	100mg/kg LE	250mg/kg LE	500mg/kg LE	Positive Control	Negative Control
4	16±1.2	14±3.5	14±3.1	12±1.8	19±3.3
5	14±2.4	11±1.7	10±2.9	10±1.4	22±4.1
6	14±1.7	10±1.5	9±2.5	8±2.4	25±2.8
7	12±1.8	10±2.1	7±1.3	5±1.1	28±2.5

Data represent Mean ± Standard Deviation of the Triplicates

NOTE:

- *LE= Leaf extract*
- *Positive Control= 5mg of Chloroquine*
- *Negative Control= Saline Water*

Figure 1 shows the suppressive activity (percentage inhibition) of the leaf extract of *I. gabonensis* in the erythrocytes of *P. berghei* infected Swiss Albino mice. On treatment with 100mg/kg of the leaf extract of *I. gabonensis*, the percentage inhibition of the extract ranged from 15.80% as at the 1st day after extract administration to 57.20% after termination of treatment. Treatment with 250mg/kg of the leaf extract produced a percentage inhibition of 26.40% as at the 1st day after extract administration to 64.30% as at the end of treatment. On treatment with 500mg/kg of the leaf extract the percentage inhibition of the extract ranged from 26.40% as at the 1st day after extract administration to 75.0% as at the 4th day after termination of treatment. The 5mg chloroquine (Positive control) group produced a percentage inhibition of the extract ranging from 36.80% as at the 1st day after extract administration to 82.10% as at the 4th day after end of treatment.

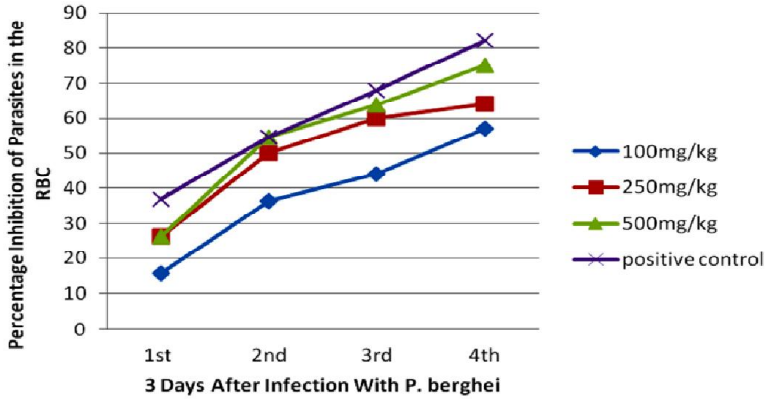

Figure 1: Percentage Inhibition of Leaf extracts of *I. gabonensis* in the erythrocytes of *P. berghei* infected Swiss Albino mice

Discussion

Plants have been reported to be used in the treatment of malaria, jaundice, diabetes and abscesses (Abdulelah and Zainal-Abidin, 2007; Ajaiyeoba *et al*., 2006; Bhat and Surolia, 2001). These prompted the need to evaluate the in vivo antiplasmodial potentials of the crude leaf extract of *I. gabonensis*, so as to ascertain its ethnobotanical uses in the treatment of malaria. The result of the preliminary phytochemical screening showed the presence metabolites of interest such as alkaloids, tannins, glycosides, flavonoids, steroids, saponins, steroids, resins and carbohydrates. Some secondary metabolites of plants are said to have antiplasmodial activity (Dikasso *et al*., 2006; Okokon *et al*., 2005). In addition, *I. gabonenis* has been reported to contain monoterpenes which have been implicated in antiplasmodial activities of plants. Phytochemical compounds such as alkaloids and terpenes and their derivatives such as monoterpenes have been implicated in antiplasmodial activity of many plants. Monoterpenes such as limonene have been implicated in endoperoxidation leading to plasmocidal activity (Clarkson *et al*., 2004). These could have also contributed to the antiplasmodial activity of this extract. Some plants are known to exert antiplasmodial activity either by causing red blood cell oxidation or by inhibiting protein synthesis depending on their phytochemical constituents (Dikasso *et al*., 2006; Okokon *et al*., 2005; Abdulelah and Zainal-Abidin, 2007; Ajaiyeoba *et al*., 2006; Bhat and Surolia, 2001).

The results obtained in Table 2 showed that the extract significantly ($P \geq 0.05$) reduced the parasitaemia count in the erythrocytes of *P. berghei* infected albino rats in a dose dependent fashion. The highest dose of the extract (500mg/kg) exhibited higher antiplasmodial activities than the lower doses of 100mg/kg and 250mg/kg respectively. This could be attributed to increase in the concentration of the bioactive compounds in the higher doses of the extract (Okokon *et al*., 2005). The extract of *I. gabonensis* leaves showed antimalarial activity against *P. berghei* infection in mice as evidenced by percentage of parasite inhibition (Figure, 3).The effects on parasitaemia in this study are similar to the one reported by previous study such as that of *Asparus africanus* (Bhat and Surolia, 2001). However, relatively higher antiplasmodial activities than the present results have been reported on *Casuarina equistifolia*, *Mangifera indica*, *Terminalia catappa*, *Nigella sativa*, and *Azadirachta indica* (Malann *et al*., 2013; Madara *et al*., 2011; Abdulelah and Zainal-Abidin, 2007; Ajaiyeoba *et al*., 2006).

Furthermore, it was observed that the antiplasmodial activity of the doses increases as the duration of the experiment progresses. It is worthwhile to note that as the day progresses, the plant extract increases the red blood cell oxidation as well as inhibiting protein synthesis as this causes decrease in parasitaemia (Table, 2). The results of this study demonstrated that crude leaf extract of *I. gabonensis* possesses considerable antiplasmodial activities and has confirmed the use of this plant to treat malaria and related symptoms in folkloric medicine. In conlusion, the results obtained from this study authenticate the rationale and continous use of this experimental plant traditional therapy of malaria in tropical regions of Africa.

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