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In vivo Schizonticidal Activity of Ethanolic Leaf Extract of *Gongronema Latifolium* on *Plasmodium Berghei Berghei* in Mice

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Abstract

In vivo schizonticidal activity of ethanolic leaf extract of *Gongronema latifolium* on blood-borne chloroquine-sensitive *Plasmodium berghei berghei* in mice was determined so as to scientifically justify the traditional use of the plant in south eastern Nigeria (tropical rain forest region) for local management of malaria fevers. The ethanolic leaf extract of *Gongronema latifolium* (200 – 800mg/kg) was administered orally to mice during early and established *Plasmodium berghei berghei* infections and its repository action in blood was also determined. The leaf extract at these doses caused 71-81% inhibition of parasitemia in the suppressive test, 59-73% parasitemia inhibition in the repository test and a mean survival time of 25-29 days in the curative test. These results show significant ($P<0.05$) antiplasmodial activity in the four-day suppressive test and in the curative test. These findings support the traditional use of the leaf extract of *Gongronema latifolium* for local treatment of malaria.

Keywords: Antimalarial activity, *Plasmodium berghei*, *Gongronema latifolium*, Herbal medicine.

Introduction

Malaria constitutes one of the major public health problems in tropical Africa. It is estimated that over 250 million Africans are infected by malaria parasites with nearly 90 million clinical cases annually. Deaths occurring from this African endemic disease have increased to an estimate of two million every year (1). *Plasmodium falciparum*, the most widespread etiological agent for human malaria has been reported to be increasingly resistant to standard antimalarial drugs and this situation necessitates a continued effort to search for new drug entities, particularly with novel modes of action (2).

In Africa, the use of indigenous plants still plays an important role in traditional management of malaria fevers (3). These indigenous plants have served as interesting leads for the discovery of novel antiplasmodial

compounds. *Gongronema latifolium* (known as Utazi in the South Eastern and Arokeke in the South-Western parts of Nigeria) is a tropical rainforest plant which belongs to the family Asclepiadaceae (4, 5). It is a climber with tuberous base and it is also found in deciduous forests of Guinea Bissau and Western Cameroons. Various parts of this plant, particularly the stems and leaves are used as chewing sticks or liquor in places such as Sierra Leone. The liquor, usually obtained after the plant is sliced and boiled with lime juice or infused with water over three days is usually taken as a purge for colic and stomach pains. It is also used to treat symptoms related to worm infections (6).

Gongronema latifolium is used in South-Eastern Nigeria to treat various ailments such as cough, loss of appetite, malaria and stomach disorders. The plant is also used as a spice and as a soup vegetable (7) for maintaining healthy blood glucose levels (8). Use of medicinal plants in traditional management of diseases is as old as man (9). An antibacterial activity of the leaf extract of this plant has also been reported (10). The South-Eastern Nigeria geographic location falls within the tropical rain forest where biodiversity includes a large population of plants, mushrooms, riverine and oceanic animals.

The World Health organization (WHO) has long recognized and encouraged many countries to exploit their flora and fauna for the ever increasing interest of the public in the use of medicinal plants and their products in the treatment of various ailments. These plants which are found mostly in local communities have been acceptable to the population and serve as a cheaper alternative to orthodox medicine (11,12). On the basis of traditional Nigerian use as an antimalarial plant medicine, our intent in this study was to scientifically evaluate the ethanol extract of *Gongronema latifolium* possibly for wider acceptability as a malarial remedy.

Materials and methods

Plant materials

Fresh leaves of *G. latifolium* were harvested from the woods of Ihiagwa, Owerri, Imo State, Nigeria, in May, 2009. The plant was identified and authenticated by Mrs. Grace Ugbabe of Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. A voucher specimen (No. NIPRD 6395) was deposited at the herbarium unit of the Institute for future reference.

Plant extraction

The dried and powdered leaves of *G. latifolium* (250g) were extracted by maceration with ethanol for three consecutive days at room temperature together with constant shaking. The liquid extract obtained was concentrated to dryness by vacuum at 40°C. The yield was calculated to be 2.8%.

Animals

Albino Swiss mice (18-22g) of either sex obtained from Animal Facility Centre (AFC), National Institute for Pharmaceutical Research and Development (NIPRD) were used for the study. The animals were kept in cages at room temperature and moisture, under naturally illuminated environment of 12:12 hour dark/ light cycle. The animals were used in accordance with NIH Guide for the care and use of laboratory animals (13).

Phytochemical Screening

Standard screening tests of the extract were carried out for various plant constituents.

The ethanol extract was screened for the presence of alkaloids, flavanoids, saponins and tannins using standard procedures (14).

Acute toxicity test (LD₅₀ determination)

The LD₅₀ of the extract administered orally in albino mice was determined using Locke's (15) method. Dose levels used ranged from 100-5000mg/kg body weight of the leaf extract. The acute toxicity LD₅₀ was calculated as the geometric mean of the dose that resulted in 100% lethality and that which caused no lethality at all. Toxicity signs such as death, changes in physical appearance and behavioral changes were observed for four days as part of our studies.

Parasite Inoculation

The chloroquine-sensitive *plasmodium berghei berghei* was obtained from Animal Facility Centre, National Institute for Pharmaceutical Research and Development, Abuja, where the parasites are maintained through weekly passage in mice. The inoculums consisted of *P. berghei berghei* parasitized erythrocytes. This was prepared by determining both the percentage parasitemia and the erythrocytes count of the donor mouse and diluting the blood with normal saline in proportions indicated by both determinations. Each mouse was inoculated on day 0, intraperitoneally with 0.2ml of infected blood containing 1×10^7 *P. berghei berghei* parasitized red blood cells.

Evaluation of blood schizonticidal activity on an early infection (4 days test)

This test was performed in a four day suppressive standard test using previously reported methods of (16,17). Thirty Swiss albino mice of either sex weighing (18 - 22g) were inoculated by interaperitoneal (i.p) injection with infected erythrocytes (0.2ml) containing 1×10^7 *P. berghei berghei* parasitized erythrocytes. The animals were divided into five groups of six mice each. They were orally administered with 200, 400 and 800mg/kg/ day doses of the *Gongronema latifolium* leaf extract, chloroquine 10mg/kg/day and an equivalent volume of tween 80 (negative control) for four consecutive days (day 0 to day 3). On the fifth day (day 40), thick films were made from the tail blood of each mouse and fixed with methanol, stained with Giemsa and parasitemia was determined by manually counting the parasitized red blood cells on at least 1000 red blood cells.

The percentage suppression of parasitemia was calculated for each dose level by comparing the parasitemia in infected controls with those of treated mice.

Evaluation of the repository activity

The repository activity was determined using the method described by (18). In this method, the mice were divided into five groups of six mice each in a cage and the animals were administered 200, 400 and 800mg/kg/day dose of the extract, 10mg/kg/day chloroquine (positive control) and tween 80 (negative control) for 4 days (D0-D3). On the fifth day (D4), the animals were inoculated with plasmodium berghei berghei. Seventy-two hours after, the parasitemia level was determined by thick blood smears as described above.

In vivo chemosuppressive test

The 4-day suppressive test against *P. berghei* infection in mice was modified and employed (19) in our studies. Parasitized erythrocytes were obtained from a donor-infected mouse by cardiac puncture using a sterile needle and syringe. Thirty mice were selected and inoculated intraperitoneally with infected blood suspension (0.2ml) containing 1×10^7 infected erythrocytes. Mice were randomly divided into five groups, with groups I, II and III receiving daily doses of the extract by oral route (200, 400, 800mg/kg), group IV (negative control) received equal volume of normal saline, while group V (serving as positive control) was treated with chloroquine at a total dose of 10mg/kg p.o., all on the day one.

This treatment was continued daily until the fourth day. On the fifth day (post day 4 of treatment) blood was collected

from the tail of each mouse and thick films fixed with methanol, stained with 4% Giemsa at pH 7.2 for 30 min were prepared. Parasitemia was determined by counting the parasitized red blood cells out of 1000 red blood cell in 10 random microscopic fields.

Percentage growth inhibition of the parasites was calculated by the following formula:

Growth inhibition (%) =

$$\frac{((\text{Parasitemia in negative control} - \text{parasitemia in study group}) / \text{Parasitemia in negative control}) \times 100}{}$$

Statistical analysis

Data obtained from the study were expressed as mean \pm standard error of mean (S.E.M). The data was analyzed using students t-test and differences between means were considered significant when $p \leq 0.05$ (20).

Results

Phytochemical Tests

The ethanol extract of *Gongronema latifolium* gave a positive reaction for each of the following secondary metabolites: alkaloids, saponins , tannins and flavanoids.

Acute toxicity tests

No mortality was observed in the mice, even in doses as high as 500mg/kg p.o. This indicates that the doses used are relatively safe.

For-day test

The ethanolic leaf extract of *G. latifolium* produced a dose dependent chemosuppressive activity at different doses employed. Doses of 200, 400 and 800mg/kg/day caused chemosuppression of 71%, 76% and 81% respectively (table 1). The standard drug, chloroquine 10mg/kg/day, caused 88% suppression which was a significantly ($P < 0.05$) higher chemosuppression than that produced by the extract treated groups.

Prophylactic effect

The ethanolic extract of *G. latifolium* exhibited a dose dependent prophylactic activity at the various doses employed. Extract doses of 200, 400 and 800mg/kg/day produced 59%, 65% and 74% chemosuppression respectively (table 2). However, the standard drug chloroquine (10mg/kg/day) exhibited a considerably higher (85%) chemosuppressive activity than the extract treated groups.

Curative effect

In established *P. berghei berghei* infection in rats, it was

observed that there was a daily increase in parasitemia in the control group. However, there was also a dose-dependent reduction in parasitemia levels in the extract-treated groups, similar to the reduction in parasitemia observed in the chloroquine treated group (table 3).

In summary, figure 1 shows a graphical representation of the relative antimalarial effectiveness (calculated as

degrees of antimalarial effectiveness were observable for 800mg/kg of ethanolic extract of *Gongonema latifolium* and 10mg/kg of chloroquine.

Discussion

As shown from the results of the in vivo antiplasmodial studies presented in table 1, the ethanolic leaf extract of *Gongronema latifolium* exhibited various degrees of antimalarial activity in chloroquine sensitive mice. One of

Table 1: Antiplasmodial activity of *G. latifolium* during suppressive test

Drug/extract	Dose (mg/kg)	Parasitemia count	% Inhibition
Control (Tween 80)	0.2ml	39.20±0.74	-
<i>G. latifolium</i>	200	11.25±1.35	71%
	400	9.43±0.39*	76%
	800	7.26±0.41*	81%
Chloroquine	10	4.70±0.39*	88%

Values are expressed as mean±Standard Error. * $p < 0.05$ when compared with values for control.

Table 2: Antiplasmodial activity of *G. latifolium* during repository test

Drug/extract	Dose (mg/kg)	Parasitemia count	% Inhibition
Control (Tween 80)	0.2ml	45.20±0.46	-
<i>G. latifolium</i>	200	18.50±2.22	59%
	400	15.66±0.95*	65%
	800	11.90±0.86*	73%
Chloroquine	10	6.33±0.39*	85%

Values are expressed as mean±Standard Error. * $p < 0.05$ when compared with values for control.

percentage activity) against of a dose of 800mg/kg of ethanolic extract of *Gongonema latifolium* and 10mg/kg of chloroquine using *Plasmodium berghei berghei* in mice. For suppressive, repository and curative tests, similar

the challenges of antimalarial drug treatment has become a highly reduced sensitivity of the plasmodium parasite to chloroquine which used to be a standard drug for malaria treatment. Mice used in our studies showed retained

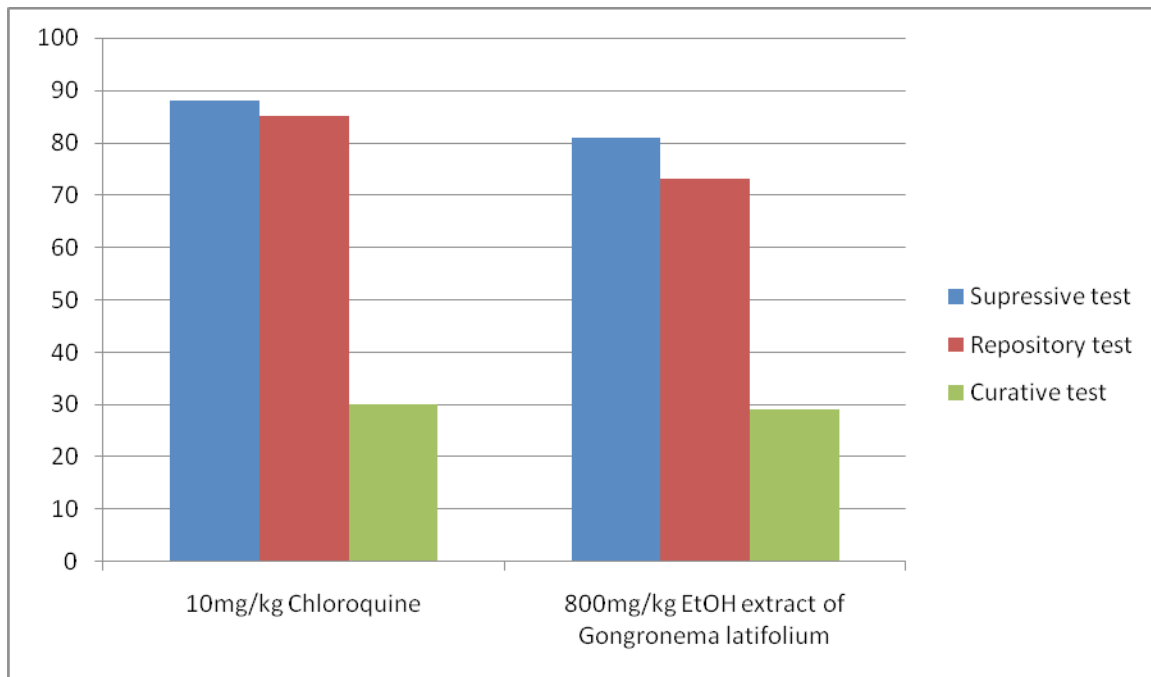


Figure 1. Histogram showing showing the relative antimalarial effectiveness (percentage activity) of a dose of 800mg/kg of ethanolic extract of Gongronema latifolium and 10mg/kg of chloroquine using Plasmodium berghei berghei in mice.

Table 3: Antiplasmodial activity of *G. latifolium* during curative test

Drug/extract	Dose (mg/kg)	Parasitemia count	Mean survival time (days)
Control (Tween 80)	0.2ml	40.11±0.82	9.0±2.0
<i>G. latifolium</i>	200	11.50±1.29*	25.5±1.2
	400	9.10±0.26*	28.0±0.9
	800	6.30±0.55*	29.2±0.5
Chloroquine	10	2.10±0.15*	30.0±0.0

Values are expressed as mean±Standard error, *p<0.05 when compared with values for control

sensitivity to the chemotherapeutic effects of 10mg/kg chloroquine. The plant extract produced significant effects in both suppressive and curative antiplasmodial tests. This ethanolic leaf extract of *G. latifolium* also exhibited significant repository activity in comparison to the standard experimental drug, chloroquine at a dose of 10mg/kg as demonstrated in the mean survival time of the mice in extract and chloroquine treated groups.

This observed antimalarial activity is consistent with the traditional use of the plant as herbal medication against the disease and indicative of its potential as a chemotherapeutic antimalarial agent. Some naturally occurring plants possessing suppressive activity against *P. berghei berghei* have been reported to exhibit antimalarial activity (21). Although the mechanism of action of this extract has not been elucidated, some plants are known to exert antiplasmodial

activity either by causing red blood cell oxidation (22) or by inhibiting protein synthesis (23) depending on their phytochemical constituents. It is possible that the ethanolic leaf extract of *G. latifolium* could have exerted its antimalarial action through either of these two mechanisms or by some other yet unknown mechanism.

G. latifolium leaf extract has been reported to contain alkaloids, flavonoids, tannins and saponins (10,24). In a report of their studies on the antibacterial properties of *G. latifolium*, Nwinyi et al showed that alkaloids, flavonoids, tannins and saponins were common phytochemical constituents of parts of this plant material obtainable in Nigeria. Previous reports of antiplasmodial screening of plant substances have implicated alkaloids, flavonoids and terpenes (25,26). In addition, our earlier studies have shown that ethanolic leaf extract of *Gongronema latifolium* possess analgesic effect (personal data). Natural products possessing such activity were reported to provide relief to malaria patients (27) and may specifically target some organs (28) in their mechanism of action.

In the process of bioprospecting for potentially effective antimalarial compounds, relative effectiveness of new antimalarials would normally be compared with standard or previously existing natural products or with those products of combinatorial chemistry. Here, we calculated percentage activity in the suppressive, repository and curative tests for both chloroquine and for the extract of *G. latifolium* (figure 1) to observe relative effectiveness as anti *Plasmodium berghei berghei* malaria. Although there was a wide variation in the doses of these two agents with chloroquine showing higher potency, it is possible to do further studies with a view to finding out how to improve the potency of the ethanolic leaf extract of *G. latifolium* as a potential antimalarial agent.

The results of this study have shown that *Gongronema latifolium* leaf extract possesses antiplasmodial activity as seen in its ability to suppress chloroquine sensitive plasmodium berghei infection in the three models evaluated. Therefore, the traditional use of this plant to treat malaria in South Eastern Nigeria is based on a real anti-parasitic activity. These results could contribute to the development of potential antimalarial drugs from the Nigerian ethnobotanical diversity.

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