

Journal of Coastal Life Medicine

journal homepage: www.jclmm.com



Original article

doi: 10.12980/jclm.3.2015j5-93

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A combination of the leaves and tuber of *Icacina senegalensis* A. Juss (Icacinaceae) improves the antimalarial activity of the plant in mice

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ARTICLE INFO

Article history:

Received 24 Jun 2015

Received in revised form 9 Jul 2015

Accepted 23 Aug 2015

Available online 28 Sep 2015

Keywords:

Icacina senegalensis tuber

Antimalaria

Mice

Plasmodium berghei

Chemosuppression

Curative

ABSTRACT

Objective: To investigate the possibility of increased antimalarial activity of *Icacina senegalensis* A. Juss (Icacinaceae) upon a combination of its leaves and tubers against *Plasmodium berghei* malaria in mice.

Methods: Chloroquine sensitive ANKA clones of *Plasmodium berghei* were used to develop experimental models based on intraperitoneal injection of 10^7 parasitized erythrocytes in phosphate buffer saline (pH 7.2) and subsequent development of parasitemia. The models were employed to investigate prophylactic and curative anti-malarial activities of tuber and tuber-leaf methanol extracts of the plant at selected dosages (25, 50 and 100 mg/kg body weight). Chloroquine with a curative dosage of 10 mg/kg body weight was used as positive control in both studies.

Results: Tuber and tuber-leaf extracts produced a dose-dependent chemosuppression of the parasites, with higher activity and mean survival time exhibited by the combined extract.

Conclusions: Anti-plasmodia activity has been discovered in methanol extract of *Icacina senegalensis* tuber extract. The observed optimization of the antimalarial actions of the plant upon a combination of its leaf and tuber opens a new area of medicinal plant research.

1. Introduction

Medicinal plants present themselves as a rich source of antimicrobial agents, and for many years, their use in health care delivery has remained the first choice for many countries in sub-Saharan Africa due to their affordability and availability. Plants have over the years proven their value as a good source of lead compounds for drug development[1,2]. Despite recent advances in malarial research and treatment, multiple resistance of the parasite to existing drugs continues to emerge, largely due to the indiscriminate use of these commercial antimalarial drugs. This has necessitated the search for new effective and affordable antimalarial agents to replace existing ones as a public health priority especially in Africa[3-6]. Globally, there is increased interest among researchers in the screening of medicinal plants to not only establish their antimalarial potential, but also identify the compounds responsible

for their activity[7-9].

Icacina senegalensis A. Juss (Icacinaceae) (*I. senegalensis*) is a shrubby perennial plant with a large underground fleshy tuber with high starch content[10]. It grows wild in the savannah areas of West Africa including Nigeria, Benin, Central African Republic, Congo, Chad and parts of Sudan[11]. In these regions, various parts of the plant, particularly the leaves and roots are widely used in traditional medicine. Decoction of the leaves is used to treat feverish conditions and as antidote for snake bite, the heated leaves as analgesic for elephantiasis, the leaf sap to treat eye infections, and water or alcohol extracts of the leaves as antihyperglycemic agents[12,13]. Scientifically, the antimalarial activity of the leaves extracts of the plant has been recently confirmed in both *in vitro* and *in vivo* antimalarial studies[13,14]. Although the tubers of the plant have been reported to be used as carbohydrates source in some parts during famine[10], there is no scientific documentation on its use in traditional medicine.

In the present study, methanolic extract of *I. senegalensis* tuber was screened for *in vivo* antimalarial activity in mice, with the secondary aim of investigating the possibility of an increased activity upon combination of the tuber with the leaves of the same

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plant.

2. Materials and methods

2.1. Plant material

I. senegalensis were uprooted and collected from a site near secretariat of Orlu Local Government Area, Imo State, Nigeria and were taken to the herbarium of Botany Department, University of Calabar for authentication. This was followed by deposition of the whole plant as a herbarium collection with voucher number 0620.

The leaves and tubers were detached from the plant stems, washed to remove dirt and taken to the Chemistry Department of University of Calabar, where they were oven-dried at 50 °C for five days and ground to powder.

2.2. Tuber extraction

Ground tuber sample (400 g) of *I. senegalensis* was extracted in analytical grade methanol (BDH, England) in a Soxhlet apparatus for 24 h to obtain the tuber extract. Also a mixture of 57 g each of ground leaves and ground tubers of the plant was exhaustively extracted as described above to obtain the combined extract, the tuber-leaf extract. Both extracts were concentrated to dryness in a water bath (Memmert, Germany) and weighed. Each of the dried samples was subsequently reconstituted with 10% (w/v) Tween 80 to obtain concentrations of 25, 50 and 100 mg/kg mouse body weight and stored in sterile universal containers at 4 °C until ready for use.

2.3. Phytochemical analysis

Standard procedures were used to determine the presence of phytates, oxalates, cyanogenic glycosides, alkaloids, tannins, saponins, terpenoids, phlobatannins, flavonoids, steroids, cardiac glycosides, phenols and anthraquinones in the methanol extract of *I. senegalensis* tuber[15-17].

2.4. Acute toxicity study of the extract

In vivo acute toxicity of the methanolic extracts was determined in mice using Lorke's method with modification[18]. Groups of six starved animals were given increasing doses of test samples (10, 100, 1000 and 2000 mg/kg body weight) orally. The animals were placed under observation for 24 h. Signs of toxicity and mortality were watched for and recorded.

2.5. Drugs

Tablets of chloroquine (Evans, Nigeria) were dissolved in phosphate buffered saline (pH 7.2) to a final dose of 10 mg/kg body weight.

2.6. Animals

Inbred male and female (non pregnant) Swiss albino mice (18–25 g) were obtained from the Department of Physiology, University of Calabar. The animals were maintained under conventional conditions of 12 h light/ dark cycle in standard cages. They were fed a pellet diet (Vital Feed, Nigeria) with water *ad libitum*. The National Institute of Health Guidelines for Care of Laboratory Animals were adopted throughout the experiment[19]. After acclimatization period of 10 days, mice were randomly divided into experimental groups.

2.7. Parasite

The *Plasmodium berghei* (*P. berghei*) clone used in this study was obtained from the Department of Pharmacology, University of Calabar, Nigeria. The parasite is an ANKA clone with chloroquine-sensitive phenotype and was maintained by serial passage in mice without chloroquine pressure.

2.8. Parasite inoculation

Each mouse was infected with a standard inoculum of 10^7 parasitized erythrocytes in phosphate buffered saline (0.2 mL) prepared from the donor mouse erythrocytes according to the method reported by Iwalokun[20].

2.9. Prophylactic treatment

A classical 4-day suppressive test according to Peters[21] and as described by Ramazani *et al.*[22] and Akuodor *et al.*[23] was used. The animals were divided into 10 groups of six mice each and treatments commenced immediately after inoculation (Day 0) and continued on Days 1, 2 and 3 via an oral route using a cannula. Animals in Groups 1a and 1b were given the vehicle only (*i.e.* 0.2 mL Tween 80) and served as the negative control. Animals in Groups 2a 2b and 2c were administered with the tuber extract in dosages of 25, 50 and 100 mg/kg body weight respectively, while those in Groups 3a 3b and 3c received the tuber-leaf extract in dosages of 25, 50 and 100 mg/kg body weight respectively. Animals in Groups 4a and 4b were given chloroquine (10 mg/kg body weight) throughout the treatment period and served as the positive control.

Stained (10% Giemsa) tail blood films of the infected animals were examined microscopically with 1000× magnification under oil immersion on Day 4 post-inoculation. Parasite density was determined by counting the number of parasitized erythrocytes in at least 10 different fields while percentage reduction in parasitemia relative to chloroquine treatment and negative control on group by group basis was calculated.

2.10. Curative treatment

A modification of the method earlier described by Ryley and Peters[24] as described by Akuodor *et al.*[23] was used. Another set of 60 albino mice randomized into 10 groups ($n = 6$) were intraperitoneally infected with 10^7 parasitized erythrocytes on the 1st day of the experiment (Day 0) with oral treatments commencing on Day 3 post inoculation (*i.e.* 72 h later) until Day 6. Animals in Groups 1a and 1b were given the vehicle only (Tween 80) and served as negative control. Animals in Groups 2a, 2b and 2c were respectively administered with the selected 25, 50 and 100 mg/kg body weight doses of the tuber extract; while the Groups 3a, 3b and 3c animals were treated with the tuber-leaf extract in dosages of 25, 50 and 100 mg/kg body weight respectively. A curative dose of standard drug (chloroquine, 10 mg/kg) was administered to Groups 4a and 4b daily for four days. Tail blood films were prepared on Day 3 post inoculation before treatment and again on Day 7 of the study at the end of treatment. Level of parasitemia reduction was determined as previously described and the number and percentage of survivors by Day 30 were also recorded. The mean survival time for each group was determined arithmetically by finding the average survival days of the mice (post-treatment) in each group over a period of 30 days (the study period).

2.11. Statistical analysis

Results obtained were expressed as mean ± SEM. The data were analyzed for significance of disparity using One-way ANOVA and the Dunnet's *post hoc* test. The $P < 0.05$ and $P < 0.01$ were regarded as significant[25].

3. Results

3.1. Phytochemical composition of methanol tuber extract

The result of phytochemical screening of *I. senegalensis* tuber extract revealed the presence of alkaloids, phenols, phytates and oxalates. Tannins, saponins, terpenoids, phlobatanins, flavonoids, steroids, cardiac glycosides, anthraquinones and cyanogenic glycosides were not detected.

3.2. Acute oral toxicity of the crude extracts

The extracts (10–2000 mg/kg) did not produce any physical signs of toxicity such as writhing, gasp, palpitation, hair erection, decreased motor and feeding activities, decreased respiratory rate, body and limb tone, and death at the test dosages. All the mice treated with 2000 mg/kg dosage of both extracts survived, therefore the oral LD₅₀ of the extracts in mice was estimated to be > 2000 mg/kg/body weight.

3.3. Chemosuppressive antimalarial test

Results of *in vivo* chemosuppressive antimalarial assays of graded doses of methanolic extract of *I. senegalensis* tuber and the tuber-leaf combination using *P. berghei* infected mice are summarized in Table 1. There was a dose dependent increase in activity (% suppression of parasitemia) with 39%, 48%, and 56% accompanying the administration of tuber extracts at 25, 50, and 100 mg/kg, respectively. Similar values (%) for *I. senegalensis* tuber-leaf extract were 70%, 76%, and 87% for the respective doses. The standard drug chloroquine (10 mg/kg) produced 93% and 92% suppression in the tuber extract and tuber-leaf extract groups respectively. All the extracts and chloroquine caused significant ($P < 0.05$) reduction in parasitemia compared to the untreated control group.

Between treatment groups, tuber-leaf extract demonstrated a noticeably higher potency than tuber extract did.

Table 1

Suppressive effect of methanolic extracts of *I. senegalensis* on *P. berghei* in mice.

Drug and dosage	Tuber extract		Tuber-leaf extract	
	No. of parasites	% Suppression	No. of parasites	% Suppression
Tween 80 (0.2 mL)	33.52±0.91	-	32.83±0.53	-
Extract (25 mg/kg)	20.47±0.52	39*	9.80±0.57	70*
Extract (50 mg/kg)	17.58±0.64	48*	7.75±0.24	76*
Extract (100 mg/kg)	14.90±0.38	56*	4.43±0.78	87**
CQ (10 mg/kg)	2.18±0.29	93**	2.75±0.29	92**

Results are expressed as mean ± SEM ($n = 6$). CQ: Chloroquine; *: $P < 0.05$ when compared with control; **: $P < 0.01$ when compared with control.

3.4. Curative antimalarial activity

Chemosuppression of parasitemia and mean survival time of the

experimental animals were used as measures of efficacy of the test extracts/ drug. The curative effectiveness of the plant extracts were also dose dependent (Table 2).

Doses of 25, 50, and 100 mg/kg of tuber extract resulted in 40%, 44%, and 51% parasitemia suppression respectively, whereas the same doses of tuber-leaf extract led to 69%, 77%, and 81% parasitemia suppression respectively. All the extracts treated groups showed significant ($P < 0.05$) chemosuppression compared to the untreated group. A similar significant trend was observed for the tuber and tuber-leaf extracts which caused an impressive 22–30 days extension of mean survival time of the test groups as against an average of 13 days recorded for the control group. As with the suppressive test, all the tuber-leaf extract doses demonstrated a much better antimalarial potency profile than tuber extract did. It is interesting to note that the mean survival time of all doses of tuber-leaf extract treated groups (26.50–29.50 days) was similar to that of the standard drug treated group (30 days).

Table 2

Anti-plasmodial effect of chloroquine (10 mg/kg) and selected doses of tuber extract and tuber-leaf extract in mice.

Curative experiments	No. of parasites		% Suppression	Mean survival time (days)
	Pre-treatment (Day 3)	Post-treatment (Day 7)		
Infected control (Tween 80)	33.12±1.02	41.45±0.59	-	13.17±1.42
TB (25 mg/kg)	31.38±0.26	25.13±0.63	40*	22.67±1.91
TB (50 mg/kg)	28.95±0.57	23.47±0.60	44*	24.33±1.86
TB (100 mg/kg)	29.62±0.28	20.65±0.57	51*	27.17±1.33
TBLF (25 mg/kg)	31.40±0.25	12.72±0.50	69*	26.50±2.22
TBLF (50 mg/kg)	30.47±0.58	9.53±0.59	77*	28.17±1.22
TBLF (100 mg/kg)	29.88±0.31	7.97±0.23	81**	29.50±0.34
CQ (10 mg/kg)	30.33±0.44	2.45±0.35	94**	30.00±0.00

Results are expressed as mean ± SEM ($n = 6$). TBLF: Tuber-leaf extract; TB: Tuber extract; CQ: Chloroquine; *: $P < 0.05$ when compared with control; **: $P < 0.01$ when compared with control.

3.5. Percentage chloroquine activity of the extracts

It was necessary to compare the antimalarial potencies (parasitemia inhibition) of tuber extract and tuber-leaf extract to that of the standard drug chloroquine (taken as 100%). The results of both chemosuppressive and curative studies are shown on Figures 1 and 2 respectively, where tuber-leaf extract proved better potency compared to tuber extract.

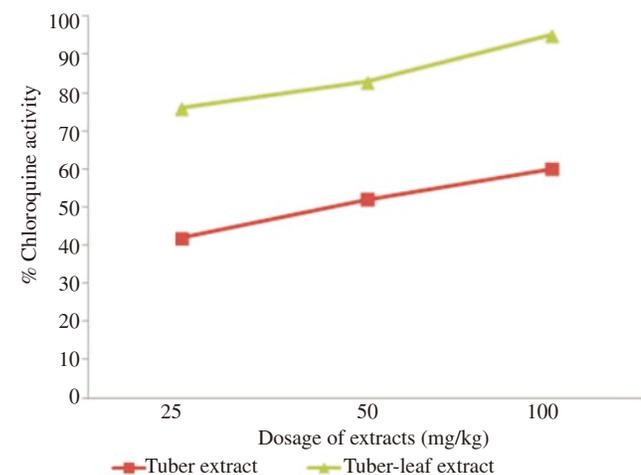


Figure 1. Comparison of chemosuppressive antimalarial activities of tuber extract and tuber-leaf extract.

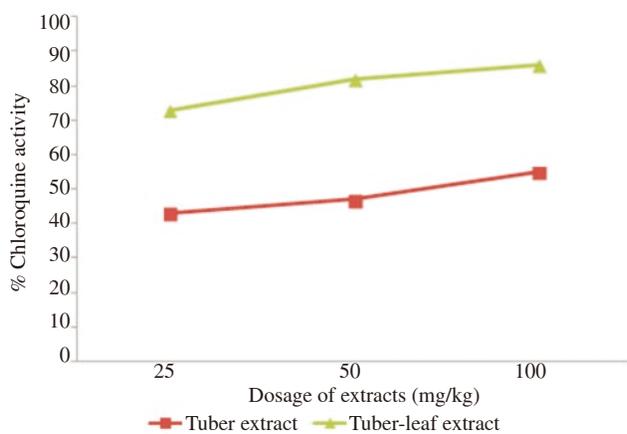


Figure 2. Comparison of curative antimalarial activities of tuber extract and tuber-leaf extract.

4. Discussion

Presently, the ineffectiveness of existing anti-malarial drugs due to drug resistance in many endemic regions of Africa has made the disease a persistent major public health problem in spite of huge investments in eradication programmes[26-28]. Due to the popularity of plants in the area in the treatment of the disease, it is necessary for academic and nonprofit institutions, in partnership with the pharmaceutical industry, to explore more plants for new antimalarial agents and to optimize those with existing antimalarial activity[29,30]. The present study has validated the antiplasmodial activity of the tuber extract of *I. senegalensis* in addition to demonstrating the potential of the plant to optimize its *in vivo* antiplasmodial activity when its leaves and tubers are extracted and administered in combination. This is seen in the 4-day suppressive assay, where the administration of the tuber-leaf extract resulted in the dose-dependent suppression of parasitemia that was far greater than the effects of equivalent doses of methanol tuber extracts when used singly in *P. berghei* infected mice up to the dose of 100 mg/kg. These combinations, particularly the tuber-leaf extract at 100 mg/kg in the curative study inhibited parasitemia due to *P. berghei* with prophylactic efficacy comparative to curative dosages of chloroquine (10 mg/kg). Meanwhile, the efficacy of the plant's methanol leaf extract has been reported by David-Oku *et al.* in an *in vivo* study[14]. The extract was found to exhibit prophylactic activity against chloroquine-sensitive *P. berghei* at low dose levels, in both the 4-day prophylactic and the 30-day curative models. This present study is an improvement on their work, as a combination of the leaves and tuber extract of the plant has been shown to have an additive effect on the antiplasmodial activity of the plant at low dose levels.

Moreover, the enhanced efficacy of a medicinal plant by a combination of the same plant parts is not a popular notion. Many parts of the various medicinal plants identified have been used in the traditional prophylaxis of various ailments, but they are hardly used in combination to treat a common ailment[31]. Meanwhile, a few recent studies have reported the *in vivo* antimalarial efficacy of some plants applied in combination. One of such works is the study of Okokon *et al.*,[32] which showed that *Hippocrata africana* and *Uraria chamae* are efficacious against *P. berghei*, but at very large

doses of 200–900 mg/kg. Gravito *et al.*[33] had previously reported a 66% parasitemia suppression (*P. berghei*) by alkaloid fraction (250 mg/kg) of *Abuta grandifolia*. Apart from their high dose-anti-*P. berghei* efficacy, these were entirely different plants and were also noted for toxicity[32-34]. The acute toxicity of tuber extract (2000 mg/kg) reported in this study is similar to that reported for the leaves of *I. senegalensis*[14]. This provides a much wider safety margin for the use of the tested parts of this plant in malarial treatment. Hence this work redefines the concept of plant-parts combination therapy in traditional medicine and opens up a new area in medicinal plants research.

It is of interest to know that the higher therapeutic efficacy observed in the tuber-leaf extract of *I. senegalensis* is traceable to antiplasmodial phytoconstituents that have been previously reported in other plants[35,36]. These include alkaloids, tannins, flavonoids and saponins. Surprisingly apart from alkaloids, phenols, phytates, and oxalates, most of these phytonutrients are absent in methanol tuber extract of the plant. This suggests that the concomitant extraction and attendant interaction of the phytonutrients in both plant parts could have produced a novel phytocompound mix that is more efficacious against the parasite than the traditional single part extracts. Also the ability of *I. senegalensis* tuber-leaf methanol extract to elicit similar antiplasmodial activity to that of chloroquine suggests that they both may share similar mechanisms of action against *P. berghei*.

Antimalarial potency has been discovered in the tuber of *I. senegalensis*. The present study has demonstrated the effectiveness of this plant in combating malaria, especially when its leaves and tubers are taken in combination. Further research on the plant for the purpose of antimalarial drug development and design would no doubt provide cost effective strategy for malaria control, especially in resource-poor countries where affordability and accessibility to orthodox medicines constitute barriers to health care[3,36-39]. Further studies to isolate, identify and characterize the active principles in the extracts are ongoing.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are grateful to Mr. Akuodor GC of Department of Pharmacology and Therapeutics, Faculty of Medicine, Ebonyi State University, Abakaliki, Nigeria for technical assistance.

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