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Further antiplasmodial effects of the aqueous extract of cymbopogon citratus stapf (lemon grass) against plasmodium berghei in Swiss albino mice

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Abstract

Lemon grass (Cymbopogon citratus Stapf) is a popular alternative to western medicines for a number of conditions, including fevers, muscle soreness and superficial infections in Nigeria. In addition to its already reported suppressive effects against P. berghei infection, this study sought to determine its repository and blood schizonticidal activities in established P. berghei infection using Swiss albino mice as models. Mice weighing on average, between 15 and 25g were given 103mg/kg, 155mg/kg and 310mg/kg/day of the crude aqueous extract of cymbopogon citratus stapf, in the 4-day test, 24-hour Rane test and 72-hour Rane test. The effects of these doses of the extract were then compared with chloroquine (5mg/kg/day) and sulphadoxine/pyrimethamine (3mg/kg/day). We report an average percentage suppressive repository activity of 65.8% for the extract at a dose of 310mg/kg and a blood schizonticidal activity that increased from 68.33% in the 24-hour Rane test to 92% in the 72-hour Rane test for the same dose of extract. The crude aqueous extract of C. citratus stapf thus has significant repository and blood schizonticidal activities against established P. berghei infection in Swiss albino mice compare to that of pyrimethamine and sulphadoxine/pyrimethamine respectively.

Keywords: cymbopogon citratus stapf, antiplasmodial activity, P. berghei,

INTRODUCTION

In Nigeria, herbal remedies are popular alternatives to standard western allopathic medications for the treatment of a variety of medical conditions. Cymbopogon citratus stapf (Lemon grass) also known among the Efik, Hausa, Ibibio and Igbo of Nigeria as ikon eti, tsauri, myoyaka akara, and achara ehi respectively, is a tall perennial plant; about 2m high, that rarely flowers and whose lower leaf sheaths have a characteristic waxy bloom[1]. It has been reported to be useful in the treatment of fevers, stomach cramps and flatulence, "muscle soreness", superficial infections, nervousness as well as a general digestive aid amongst alternative medicine practitioners[1,2].

The usefulness of cymbopogon citratus stapf as an antibiotic, antifungal and antiparasitic agent has been scientifically documented. For instance, it has been reported that extracts of this plant are active against strains of staphylococcus aureus, Candida albicans and Candida krusei[3]. The anti-proliferative effect of citral, the main constituent of the oil of C. citratus stapf against all three developmental stages of Trypanosoma cruzi has also been reported[4]. The methanolic extract C. citratus stapf has been reported to have both hypoglycaemic and hypolipidaemic effects in rats[5], to protect human erythrocytes from hypotonic shock[6], and to possess free radical scav-

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enging properties[7]. Assay guided purification has further revealed that one of its active substances is geranic acid, a potent tyrosinase inhibitory agent[8].

The in vivo anti-malarial activity of this plant has also recently been reported from Cameroun by Tchoumbougnang et al 2005[9]. The said study extracted oil from fresh leaves of C. citratus stapf by hydro-distillation and determined the effects of this oil on the growth of Plasmodium berghei. The oil which contained geranial (32.8%), neral (29.0%), myrcene (16.2%) and beta-pinene (10.5%) showed significant antimalarial activity in the four-day suppressive test in mice[9].

The present study reports the blood schizonticidal activity in both early and established infections and the repository activity of the aqueous extract of cymbopogon citratus stapf on against Plasmodium berghei using Swiss albino mice as models. This is to further characterize the antiplasmodial activity of the lemon grass.

MATERIALS AND METHODS

Preparation of Aqueous Extract

Fresh leaves of cymbopogon citratus stapf were collected from the environment of the university of Port Harcourt, Nigeria and correctly identified by an academic staff of the Department of Plant Sciences and Biotechnology, Faculty of Science, University of Port Harcourt, Nigeria. Voucher specimens were deposited in the Malaria Research Unit, Department of Pharmacology, College of Health Sciences, University of Port Harcourt for future reference. The leaves were then washed in tap water to remove contaminants such as sand and other organic matter. The leaves were then air dried daily until a constant weight was obtained over three consecutive days. The dried leaves were then ground into a fine powder using an electric dry mill (Moulinex). One hundred gram of the ground powder was subsequently dissolved in 600ml of distilled water and allowed to stand for 48 hours at room temperature. The mixture was filtered into a 250 ml conical flask using Watman's filter paper number one, following which the filtrate was dried at a temperature of 30° C for 10 hours to produce a gel-like extract, which weighed 18.55g. Appropriate concentrations of the extract were then subsequently made by serial dilution with distilled water for further experimentation.

Determination of LD50

The LD50 of cymbopogon citratus stapf was estimated using 42 albino wistar rats weighing between 150 and 250g. The rats were starved overnight at the beginning of the experiment and subsequently divided into 7 groups consisting of 6 rats per group. Each group of rat was given an exponentially increasing dose of the extract, intraperitoneally beginning with 100mg/kg. The number of dead mice in each group within 24 hours was recorded. The LD50 was calculated using the arithmetic formula of Kerber[10].

Animals and Inoculation

A total of 40 Swiss albino mice weighing between 15 and 25g were used for this study. The mice and P. berghei parasites were obtained from the Nigerian Institute of Medical Research, Lagos, Nigeria. The animals were fed standard mouse cubes and given tap water ad libitum. They were allowed 6 weeks to acclimatize to the new environment.

Experimental Design

The experimental design involved three distinct experimental protocols:

Evaluation of blood schizontocidal activity in early infection (the 4 day test). This was done using a method similar to that described by Knight and Peters 1980[11]. A total of 36 mice were used for this protocol of the study. All the mice were given standard intraperitoneal inoculums of 1.02 x 105 P. berghei parasites. The mice were then divided into 6 groups with 6 mice each. Groups 1, 2 and 3 were given 103mg/kg, 155mg/kg and 310mg/kg respectively (which represented approximately one sixth, one quarter and one half of the calculated LD50, respectively). Group 4 mice were given 5mg/kg of chloroquine (May and Baker, Nigeria) intraperitonealy; group 5 mice were given 3mg/kg of sulphadoxine/pyrimethamine (Roche, Switzerland); and group 6 mice were given 0.2 ml of distilled water. The sulphadoxine/pyrimethamine and distilled water were both given orally with the aid of an inflexible oral cannula. All drugs were administered once daily for three consecutive days. On the fourth day, thick blood smears were made from blood samples obtained from the tails of the animals. The smears were stained with Giemsa stain and examined under the light microscope for the levels of parasitaemia. The average percentage parasitaemia was calculated in

comparison to control.

Evaluation of repository activity

The repository activity was determined using the method described by Peters 1965[12]. The mice were divided into 5 groups (A to E) with 6 mice each. Groups A, B and C were given 103mg/kg, 155mg/kg and 310mg/kg of the extract respectively. Group D mice were given 1.2mg/kg of pyrimethamine, while group E rats were given 0.2ml of distilled water. The extract, pyrimethamine and distilled water were administered once daily for three consecutive days with group E mice serving as controls. On the fourth day, all the animals were inoculated with 1.02 x 105 P. berghei parasites, intraperitonealy. Seventy two hours after the inoculation, thick blood smears were made and the level of parasitaemia determined as described earlier.

Evaluation of blood schizonticidal activity during established infection (Rane Test)

This was done using the method described by Ryley and Peters[13]. As with the experiment to evaluate the blood schizonticidal activity of the extract, 36 mice were inoculated with 1.02 x 105 P. berghei parasites. On the third day, following inoculation, the animals were divided into six groups. Groups 1, 2 and 3 mice were administered 103mg, 155g and 310mg/kg of the extract respectively. Group 4 mice were given 5mg/kg of chloroquine; group 5 mice were given 3mg/kg of sulphadoxine/pyrimethamine; and group 6 mice were given 0.2 ml of distilled water only and served as controls. All drugs were given once daily for three consecutive days. Blood samples were obtained 24 and 72 hours after drug administration for thick blood smears, which were examined to determine the level of parasitaemia. The mean survival time for each group within a 28 day period was determined and recorded.

Statistical Analysis

Results obtained are presented as mean ± standard error of the mean. Statistically significant difference was determined using the Student's t-test; a P value less than 0.05 was considered significant.

RESULTS

The results obtained for each experimental protocol are as presented in Tables 1 to 4.

LD50 value

The LD50 of the aqueous extract of cymbopogon citrates stapf was estimated as 621.12mg/kg

Blood schizonticidal activity of aqueous extract of C. citratus stapf in early infection (The 4 day test)

Table 1 shows the blood schizonticidal activity of various doses of C. citratus stapf, chloroquine, sulphadoxine/pyrimethamine and distilled water. The average percentage parasitaemia and percentage suppression induced by the aqueous extract of C. citratus stapf at the highest dose administered was found to be 31 ± 0.54 and 71.40 respectively. The level of parasitaemia was higher than that obtained for sulphadoxine/pyrimethamine (22 ± 2.20) and the percentage suppression induced by the extract lower, compared with sulphadoxine/pyrimethamine (79.70). The extract, at all doses administered, and sulphadoxine/pyrimethamine (3mg/kg) induced a statistically significant suppression of P. berghei in early infection ($P < 0.05$). Chloroquine at a dose of 5mg/kg caused an average percentage parasitaemia and an average percentage suppression of 84 ± 3.13 and 22.51 respectively. These differences were, however, not significant ($P > 0.05$).

Repository activity of aqueous extract of C. citratus stapf

Table 2 shows the repository activity of C. citratus stapf compared to pyrimethamine. The highest dose of C. citratus stapf caused an average percentage parasitaemia and average percentage suppression of 23.8 ± 1.45 and 65.80 respectively. Pyrimethamine at a dose of 1.20mg/kg caused an average percentage parasitaemia and average percentage suppression of 13.20 ± 0.83 and 81.03 respectively. The effects of the extract at all doses and of pyrimethamine were found to be statistically significant ($P < 0.05$).

Blood schizonticidal activity of aqueous extract of C. citratus stapf in established infection (Rane test)

Tables 3 and 4 show the blood schizonticidal activity of the extract at 24 and 72 hours respectively, after established infection with P. berghei. At 24 hours, the highest dose of the extract administered caused an average percentage parasitaemia and average percentage suppression of 38 ± 0.80 and 68.33 respectively. The corresponding values for sulphadoxine/pyrimethamine (3mg/kg) were 29.6 ± 2.00 and 75.33 respectively. The values for chloroquine (5mg/kg) were 105.44 ± 1.44 and 12.16 respec-

tively. All these values were statistically significant ($P < 0.05$). At 72 hours, the highest dose of the extract administered caused an average percentage parasitaemia and an average percentage suppression of 48.0 ± 0.82 and 92% respectively, suggesting an increase in activity of the extract at 72 hours. Similarly, sulphadoxine/pyrimethamine also showed an increased activity at 72 hours to a value of 23.6 ± 0.05 and 83.14 for average percentage parasitaemia and average percentage suppression respectively. Chloroquine showed negative average percentage suppression at 72 hours

Table 1: Blood schizonticidal activity of aqueous extract of C. citratus stapf in early infection (4 day test)

Drug/Extract	Dose (mg/kg/day)	Average percentage parasitaemia	Average percentage suppression	Test of significant difference (t-test)
C. citratus extract	310	31.0 ± 0.54	71.40	$P < 0.05$
C. citratus extract	155	36.8 ± 0.54	66.05	$P < 0.05$
C. citratus extract	103	48.4 ± 0.45	54.98	$P < 0.05$
Chloroquine	5	84.0 ± 3.13	22.51	$P > 0.05$
Sulphadoxine/Pyrimethamine	3	22.0 ± 2.20	79.70	$P < 0.05$
Distilled Water	0.2 ml/day	108.4 ± 5.07	0	0

Table 2: Repository activity of aqueous extract of C. citratus stapf

Drug/Extract	Dose (mg/kg/day)	Average percentage parasitaemia	Average percentage suppression	Test of significant difference (t-test)
C. citratus extract	310	23.8 ± 1.45	65.80	$P < 0.05$
C. citratus extract	155	45.6 ± 0.57	34.48	$P < 0.05$
C. citratus extract	103	58.8 ± 1.73	15.52	$P < 0.05$
Pyrimethamine	1.20	13.2 ± 0.83	81.03	$P < 0.05$
Distilled Water	0.2 ml/day	69.6 ± 0.67	0	0

Table 3: Blood schizonticidal activity of aqueous extract of C. citratus stapf during established infection (24 hour rane test)

Drug/Extract	Dose (mg/kg/day)	Average percentage parasitaemia	Average percentage suppression	Test of significant difference (t-test)
C. citratus extract	310	38 ± 0.80	68.33	$P < 0.05$
C. citratus extract	155	41.8 ± 0.57	65.17	$P < 0.05$
C. citratus extract	103	60.2 ± 0.66	49.83	$P < 0.05$
Chloroquine	5	105.4 ± 1.44	12.16	$P > 0.05$
Sulphadoxine/Pyrimethamine	3	29.6 ± 2.00	75.33	$P < 0.05$
Distilled Water	0.2 ml/day	120.0 ± 0.57	0	0

Table 4: Blood schizonticidal activity of aqueous extract of C. citratus stapf during established infection (72 hour rane test)

Drug/Extract	Dose (mg/kg/day)	Average percentage parasitaemia	Average percentage suppression	Test of significant difference (t-test)
C. citratus extract	310	48.0±0.82	92.00	$P<0.05$
C. citratus extract	155	53.6±0.82	61.71	$P<0.05$
C. citratus extract	103	71.6±1.52	48.85	$P<0.05$
Chloroquine	5	197.6±5.77	-41.14	$P<0.05$
Sulphadoxine/Pyrimethamine	3	23.6±0.50	83.14	$P<0.05$
Distilled Water	0.2 ml/day	140.0±5.00	0	0

DISCUSSION

It has previously been reported that extracts of C. citratus stapf possess anti-malarial properties: in that study, Tchoumbougnang et al [9] reports that oil obtained by hydro distillation of fresh leaves of this plant and administered at doses of 200, 300 and 500mg/kg produced percentage suppression of parasitaemia of P. berghei in mice of 62.1%, 81.7% and 86.6%, respectively in the 4 day test. Results from our present study for a similar 4 day test indicate that 103, 155 and 310mg/kg/day of C. citratus stapf extract produced percentage suppression of parasitaemia of 54.98%, 66.05% and 71.40% respectively. Although the values obtained from the present study are lower, but so also are the doses we administered. In addition, the previous study extracted oil by hydro-distillation from the leaves of the plant, while the present study used crude aqueous extracts of the leaves.

Furthermore, the present study reports that the crude aqueous extract of C. citratus has both significant repository activity and blood schizonticidal activity in established infection. For the repository activity, the present study reports a 65.8% suppression of parasitaemia 72 hours after administration of a 310mg/kg/day of C. citratus aqueous extract intraperitoneally; although this value is lower than the repository activity of pyrimethamine which was found to be 81.03% (Table 2). In addition, the extract also exhibited significant blood schizonticidal activity in established P. berghei infection; causing a 68.33% suppression of parasitaemia after 24 hours and 92% suppression of parasitaemia after 72 hours following a dose of 310mg/kg. Noteworthy, is that the values obtained from the extract at 72 hours are higher than that obtained for both chloroquine and sulphadoxine/pyrimethamine; however, the values obtained for the extract at 24 hours are lower than that obtained for sulphadoxine/pyrimethamine. Aside from its established suppressive effects, the results obtained from the present study apparently suggest that the antiplasmodial effect of C. citratus extract could possibly include the treatment of established P. berghei infection; as well significant repository activity.

In conclusion, the result of the present study confirms previous findings of the antiplasmodial properties and popular anti-malarial uses of C. citratus stapf in Nigeria. In addition, we report that the crude aqueous extract of C. citratus stapf has significant repository activity and blood schizonticidal activity in established P. berghei infection in Swiss albino mice.

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