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Antimalarial and analgesic activities of ethanolic leaf extract of *Panicum maximum*

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ABSTRACT

Objective: To evaluate antiplasmodial and analgesic activities of ethanolic leaf extract/fractions of *Panicum maximum*. **Methods:** The crude leaf extract (47–190 mg/kg) and fractions (chloroform, ethyl acqeous and methanol; 96 mg/kg) of *Panicum maximum* were investigated for antiplasmodial activity against chloroquine sensitive *Plasmodium berghei* infections in mice and for analgesic activity against chemical and heat–induced pains. The antiplasmodial activity during early and established infections as well as prophylactic were investigated. Artesunate at 5 mg/kg and pyrimethamine at 1.2 mg/kg were used as positive controls. Analgesic activity of the crude extract/fractions was also evaluated against acetic acid, formalin and heat–induced pains. **Results:** The extract and its fractions dose–dependently reduced parasitaemia induced by chloroquine sensitive *Plasmodium berghei* infection in prophylactic, suppressive and curative models in mice. These reductions were statistically significant ($P<0.001$). They also improved the mean survival time from 13 to 28 days compared with control ($P<0.001$).The activities of extract/fractions were incomparable to that of the standard drugs (Artesunate and pyrimethamine). On chemically and thermally–induced pains,the extract inhibited acetic acid and formalin–induced inflammation as well as hot plate–induced pain in mice. These inhibitions were statistically significant ($P<0.001$) and in a dose–dependent fashion. **Conclusions:** *Panicum maximum* leaf extract has antiplasmodial and analgesic activities which may in part be mediated through the chemical constituents of the plant.

1. Introduction

Panicum maximum. Jacq (poacace) is a perennial, tuft grass with a short, creeping rhizome regarded as the most valuable fodder plant and extensively used to make hay. The stem of this robust grass can reach a height of up to 2 m, the leaf sheath are found at the bases of the stems and are covered in fine hairs. It is widely distributed in Africa where it originates and almost all tropical parts of the world[1].The plant (leaf) is use traditionally by the Ibibios of Akwa Ibom State, Nigeria in the treatment of various ailments such as malaria, infections, rheumatism pain, inflammation and diabetes. Antia *et al*[1] had reported on the antidiabetic activity of the leaf extract. Ethnopharmacological and scientific reports on this plant is scarce. In this study, we investigated the effect of ethanolic leaf extract of *Panicum maximum* on *Plasmodium berghei* (*P. berghei*) infection in mice and experimentally–induced pains in rodents in bids to confirm its ethnobotanical uses in the treatment of various ailments.

2. Materials and methods

2.1. Plant materials

Fresh leaves of *Panicum maximum* were collected in July, 2010 at a farmland in University of Uyo, Uyo, Nigeria. The plant was identified and authenticated by Dr. Margaret Bassey, a taxonomist in the Department of Botany, University of Uyo, Uyo. Nigeria. Herbarium specimen was deposited at Faculty of Pharmacy Herbarium. The fresh leaves of the plant were dried on laboratory table for 2 weeks and reduced to powder. The powdered leaves (2 kg) was divided into two parts, one part (1 kg) was macerated in 97% ethanol(3 L) for 72 hours to give the crude ethanolic extract while the other part (1 kg) was successively and gradiently macerated for 72 hours in 3 L of each of these solvents: n–hexane, chloroform, ethyl acetate and methanol to give the corresponding gradient fractions of these solvents. The liquid filtrates were concentrated and evaporated to dryness in vacuo 40 ℃ using rotary evaporator. The yield of each extract was calculated. The dry extracts were stored in a refrigerator at –4 ℃until used for further tests.

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2.2. Animals

Albino Swiss mice (20–25 g) of either sex were obtained from the University of Uyo animal house. They were maintained on standard animal pellets and water *ad libitum*. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo.

2.3. Microorganism

A chloroquine sensitive strain of *P. berghei* berghei (ANKA) was obtained from the National Institute of Medical Research (NIMER), Lagos and was maintained by subpassage in mice.

2.4. Determination of median lethal dose (LD_{50})

The LD_{50} of the extract was estimated using the method of Miller and Tainter[2]. The animals were observed for manifestation of physical signs of toxicity such as writhing, decreased motor activity, decreased body/limb tone, decreased respiration and death. The number of deaths in each group within 24 h was recorded.

2.5. Parasite inoculation

Each mouse used in the experiment was inoculated intraperitoneally with 0.2 mL of infected blood containing about 1×10^7 *P. berghei* berghei parasitized erythrocytes. The inoculum consisted of 5×10^7 *P. berghei* berghei erythrocytes per mL. This was prepared by determining both the percentage parasitaemia and the erythrocytes count of the donor mouse and diluting the blood with isotonic saline in proportions indicated by both determinations[3,4].

2.6. Drug administration

The drugs (artesunate and pyrimethamine), extract and fractions used in the antiplasmodial study were orally administered with the aid of a stainless metallic feeding cannula.

2.7. Evaluation of antiplasmodial activity of the extract/fractions

The evaluation of suppressive activity (4-day test) of the extract, fractions and artesunate against early *P. berghei* berghei infection in mice was done with modifications as earlier described[3,4]. On the first day (D_0), the forty-eight mice were infected with the parasite and randomly divided into 8 groups of six mice each. The mice in groups 1–3 were administered with the 48, 96, and 144 mg/kg of crude extract, groups 4–6 were administered with the 96 mg/kg of the chloroform, ethyl acetate and methanol fractions respectively, while group 7 was administered with 5 mg/kg of artesunate (positive control), and 10 mL/kg of distilled water to group 8 (negative control) for four consecutive days (D_0–D_3) between 8 am and 9 am. On the fifth day (D_4), thin blood film was made from tail blood. The film was then stained with leishman stain to reveal parasitized erythocytes out of 500 in a random field of the microscope. The average percentage suppression of parasitaemia was calculated in comparison with the controls as follows:

(Average % parasitaemia in negative control–Average % parasitaemia in Positive groups)/Average % parasitaemia in negative control.

The evaluation of curative activities of extract and fractions of Panicum maximum in established infection were done with modifications as earlier described[3,4]. *P. berghei* berghei was injected intraperitoneally into another 48 mice on the first day (D_0). Seventy-two hours later (D_3), the mice was divided randomly into eight groups of six mice each and treated as above with extract, fractions and artesunate once daily for 5 days. Leishman's stained thin smears were prepared from tail blood samples collected on each day of treatment to monitor parasitaemia level. The mean survival time (MST) of the mice in each treatment group was determined over a period of 29 days (D_0–D_{28}).

$$\frac{\text{No of days survived}}{\text{Total No. of days (29)}}\times100=\text{MST}$$

Prophylactic or repository activities of extract and fractions were assessed by using the method earlier described[3,5]. The mice were treated as above for three days but 1.2 mg/kg/day of pyrimethamine was given to group 7 (positive control). On the fourth day (D_3) the mice were inoculated with *P. berghei* berghei. The parasitaemia level was assessed by blood smears seventy-two hours later.

2.8. Evaluation of analgesic potential of the extract

Acetic acid induced writhing in mice was carried out according to the procedure earlier described[6–8]. The animals were divided into 8 groups of 6 mice per group. Group 1 served as negative control and received 10 mL/kg of normal saline, while groups 2, 3 and 4 were pre-treated with 48, 96 and 144 mg/kg doses of *Panicum maximum* extract intraperitoneally, while groups 5–7 were respectively given 96 mg/kg/day of the chloroform, ethyl acetate and methanol fractions, and group 8 received 100 mg/kg of acetyl salicylic acid. After 30 minutes, 0.2 mL of 2% acetic acid was administered intraperitoneally (i.p). The number of writhing movements was counted for 30 minutes. Antinociception (analgesia) was expressed as the reduction of the number of abdominal constrictions between control animals and mice pretreated with extracts.

Formalin-induced hind paw licking in mice was carried out according to a modified method earlier described[8–10]. The animals were treated as above but injected with 20 μL of 2.5% formalin solution (0.9% formaldehyde) made up in phosphate buffer solution (PBS concentration: NaCl 137 mM, KCl 2.7 mM and phosphate buffer, 10 mM) subcutaneously under the surface of the right hind paw 30 minutes post-treatment. The amount of time spent licking the injected paw was timed and considered as indication of pain. The first phase of the nociceptive response normally peaks 5 minutes after injection and the second phase 15–30 minutes after formalin injection, representing the neurogenic and inflammatory pain responses, respectively[9]. The responses were measured for 5mins after formalin injection (first phase) and 15–30 mins after formalin injection (second phase).

For hot-plate test, the hot p-ate was maintained at (45±1) ℃. Each animal was placed into a glass beaker of 50 cm

diameter on the heated surface 30 minutes post-treatment with extract/fractions and standard drug as above. The time(s) between placement and shaking or licking of the paws or jumping was recorded as the index of response latency. An automatic 30-second cut-off was used to prevent tissue damage[6,7,11].

2.9. Statistical analysis

Data obtained from this work were analyzed statistically using Students' *t*-test and ANOVA (One- or Two- way) followed by a post test (Tukey-Kramer multiple comparison test). Differences between means will be considered significant at $P< 0.05$.

3. Results

3.1. Determination of LD_{50}

The LD_{50} was (480.00±4.91) mg/kg. The physical signs of toxicity included excitation, paw licking, increased respiratory rate, decreased motor activity, gasping and coma which was followed by death.

3.2. Suppressive activity and prophylactic activity of ethanolic crude extract and fractions of Panicum maximum

Evaluation of suppressive activity of leaf extract/fractions of *Panicum maximum* during 4-day test shows that the crude leaf extract demonstrated a dose-dependent chemosuppresive effect on the parasitemia. The ethyl acetate and methanol fractions had comparable activity. These effects were statistically significantly compared with to the control ($P<0.001$). However, ar tesunate, had a higher activity than the extract and fractions (Table 1).

Table 1
Suppressive activity of ethanolic leaf extract and fractions of *Panicum maximum* on *P. berghei* infection in mice.

Group	Dose	Parasitaemia	Chemosuppression (%)
Normal saline group	10 mL/kg	23.38±0.67	–
Panicum maximum crude extract group	48 mg/kg	8.00±1.11[a]	65.78
	96 mg/kg	6.20±3.60[a]	73.48
	144 mg/kg	6.00±1.33[a]	74.33
Chloroform fraction group	96 mg/kg	9.00±2.66[a]	61.51
Ethyl acetate fraction group	96 mg/kg	7.01±1.17[a]	70.01
Methanol fraction group	96 mg/kg	7.00±1.77[a]	70.05
Artesunate group	5 mg/kg	2.66±1.68[a]	88.62

[a]: $P<0.001$, compared with the normal saline group.

In prophylactic activity test, ethanolic crude extract showed a dose-dependent reduction of parasitaemia in the extract-treated groups. These reductions were statistically significant compared to the control ($P<0.01$). The methanol fraction demonstrated a high antiplasmodial activity which was significant ($P<0.001$) when compared to control and comparable in effect of pyrimethamine at 1.2 mg/kg(Table 2).

In curative activity test, there was a progressive dose-dependent reduction of parasitaemia in all the extract/fraction-treated group relative to control. These reductions were statistically significant compared with the control ($P<0.001$)(Figures 1 and 2). The crude extract and methanol fraction exhibited a high chemosuppression that were higher compared to that of the artesunate on day 7.

The crude extract (144 mg/kg) and fractions significantli increased MST ($P<0.001$). However, the extract and fractions were not as effective as artesunate (Table 3).

Table 2
Repository/Prophylactic activity of ethanolic leaf extract and fractions of *Panicum maximum* on *P. berghei* infection in mice.

Group	Dose	Parasitaemia	Chemosuppression (%)
Normal saline group	10 mL/kg	17.66±0.15	–
Panicum maximum crude extract group	48 mg/kg	10.67±0.51[a]	39.58
	96 mg/kg	5.36±0.67[b]	69.64
	144 mg/kg	5.00±1.93[b]	71.68
Chloroform fraction group	96 mg/kg	6.33±2.28[b]	64.15
Ethyl acetate fraction group	96 mg/kg	9.00±0.88[b]	49.03
Methanol fraction group	96 mg/kg	4.33±0.67[b]	75.48
Pyrimethamine group	1.2 mg/kg	3.66±0.93[b]	79.27

[a]: $P<0.01$, [b]: $P<0.001$, compared with the normal saline group.

Table 3
Effect of ethanolic leaf extract and fractions of *Panicum maximum* on MST(mean±SEM).

Group	Dose	MST(days)
Normal saline group	10 mL/kg	11.66±0.68
*Panicum maximum*crude extract group	48 mg/kg	13.33±0.57
	96 mg/kg	14.00±0.44
	144 mg/kg	17.67±0.93[a]
Chloroform fraction group	96 mg/kg	18.00±0.93[a]
Ethyl acetate fraction group	96 mg/kg	17.60±0.57[a]
Methanol fraction group	96 mg/kg	17.66±0.93[a]
Artesunate group	5 mg/kg	20.33±0.52[a]

[a]: $P<0.001$, compared with the normal saline group.

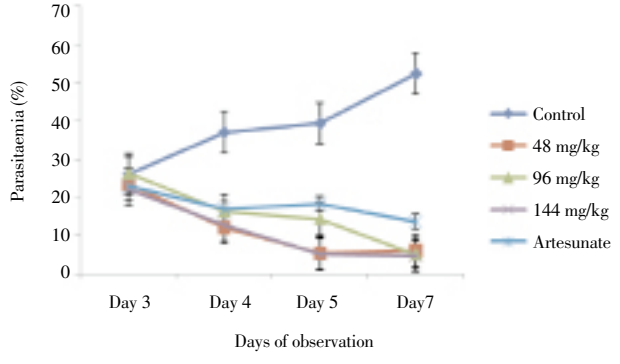

Figure 1. Curative effect of crude ethanolic leaf extract of *Panicum maximum* on *P. berghei* infection in mice.
ART – Artesunate.

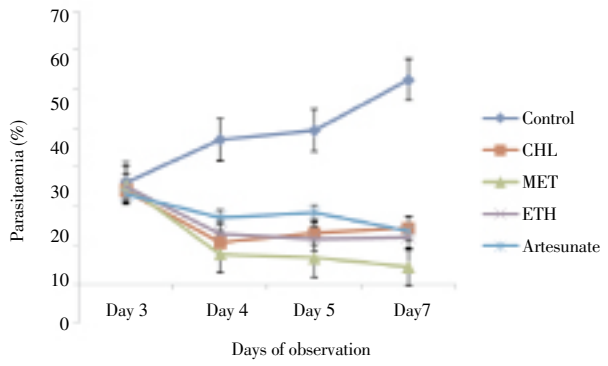

Figure 2. Curative effect of various leaf fractions of *Panicum maximum* on *P. berghei* infection in mice.
ART – Artesunate, CHL–Chloroform, ETH–Ethyl acetate, MET–Methanol.

3.3. Analgestic activity of ethanolic crude extract and fractions of leaves of Panicum maximum

The extract (48–144 mg/kg) demonstrated a dose–dependent reduction in acetic acid–induced writhing of mice. The reductions were statistically significant ($P<0.001$). The chloroform fraction exhibited the most significant analgesic activity ($P<0.001$) which was comparable to that of acetyl salicylic acid at 30 min (Table 4).

The extract exhibited a dose– dependent effect on formalin–induced hind paw licking of mice. This inhibition was significant compared with the control ($P<0.001$). Ethyl acetate fraction had the most significant effect and was comparable to that of acetyl salicylic acid at 30 min (Table 5).

The extract/fractions exhibited a dose–dependent effect on thermally–induced pain in mice. This inhibition was only statistically significant at the highest dose(144 mg/kg) compared with the control ($P<0.001$), and was uncomparable to that of acetyl salicylic acid (Table 6).

Table 6
Effect of *Panicum maximum* leaf extract on hot plate test(mean±SEM).

Group	Dose	Reaction time (sec)
Normal saline group	10 mL/kg	0.27±0.01
*Panicum maximum*crude extract group	48 mg/kg	0.33±0.03
	96 mg/kg	0.60±0.09
	144 mg/kg	1.32±0.19[a]
Chloroform fraction group	96 mg/kg	0.41±0.02
Ethyl acetate fraction group	96 mg/kg	0.59±0.17
Methanol fraction group	96 mg/kg	0.39±0.02
Acetyl salicylic acid group	5 mg/kg	2.13±0.16[a]

[a]: $P<0.001$, compared with the normal saline group.

Table 4
Effect of *Panicum maximum* leaf extract on acetic acid– induced writhing of mice(mean±SEM).

Group	Dose	Number of writhing movement						
		5 min	10 min	15 min	20 min	25 min	30 min	Total
Normal saline group	10 mL/kg	4.00±0.38	13.60±0.51	15.00±1.20	15.00±0.80	11.60±0.51	11.00±0.97	55.20±0.29
*Panicum maximum*crude extract group	48 mg/kg	4.00±0.36	6.00±0.36[a]	11.50±0.50	10.00±0.36[a]	5.66±0.49[a]	5.66±0.55[a]	42.82±2.62[a]
	96 mg/kg	3.16±0.40	5.00±0.36[a]	8.33±0.67[a]	9.33±0.76[a]	6.83±0.60[a]	2.33±0.20[a]	34.98±3.00[a]
	144 mg/kg	1.00±0.25[a]	4.66±0.33[a]	5.83±0.47[a]	3.83±0.58[a]	4.16±0.47[a]	1.83±0.30[a]	21.31±2.40[a]
Chloroform fraction group	96 mg/kg	0.83±0.30[a]	10.00±0.52[b]	10.00±0.60[a]	7.66±0.42[a]	3.00±0.52[a]	1.33±0.49[a]	32.82±2.85[a]
Ethyl acetate fraction group	96 mg/kg	0.00±0.00	9.33±0.76[b]	3.66±0.33[a]	6.00±0.85[a]	5.00±0.79[a]	3.00±0.57[a]	26.99±3.30[a]
Methanol fraction group	96 mg/kg	6.33±0.61[a]	15.5±1.38	13.00±1.34	8.33±1.45[a]	5.50±0.62[a]	3.00±0.63[a]	51.66±6.03
Acetyl salicylic acid	100 mg/kg	0.00±0.00	0.30±0.48[a]	6.66±0.76[a]	4.33±0.61[a]	4.00±0.57[a]	1.33±0.23[a]	16.62±2.65[a]

[a]: $P<0.01$, [b]: $P<0.05$, compared with the normal saline group.

Table 5
Effect of *Panicum maximum* leaf extract on formalin–induced hind paw licking of mice(mean±SEM).

Group	Dose	Number of paw licking						
		5 min	10 min	15 min	20 min	25 min	30 min	Total
Normal saline group	10 mL/kg	31.50±0.92	12.33±0.71	15.12±0.16	9.17±0.40	6.67±0.59	5.67±0.33	80.46±3.11
*Panicum maximum*crude extract group	48 mg/kg	28.70±0.61	4.83±0.60[b]	4.00±0.36[b]	4.12±0.31[a]	4.67±0.21[b]	3.67±0.21[a]	49.99±2.30[a]
	96 mg/kg	23.50±1.48[b]	4.00±0.26[b]	0.67±0.49[b]	4.00±0.26[a]	4.83±0.31[b]	3.67±0.42[a]	40.67±3.22[a]
	144 mg/kg	18.20±2.30[a]	1.50±0.50[b]	5.12±0.31[b]	4.00±0.63[a]	2.33±0.21[a]	2.17±0.31[a]	33.32±4.26
Chloroform fraction group	96 mg/kg	8.33±0.95[a]	0.00±0.00[a]	2.17±0.31[b]	1.17±0.31[a]	0.50±0.22[a]	0.00±0.00[a]	12.17±1.79[a]
Ethyl acetate fraction group	96 mg/kg	21.00±2.07[a]	4.17±0.31[b]	1.17±0.48[b]	4.50±0.89[a]	3.17±0.31[a]	3.00±0.45[a]	37.01±4.51[a]
Methanol fraction group	96 mg/kg	15.80±2.02[a]	1.83±0.48[b]	0.83±0.40[b]	3.83±0.72[a]	2.33±0.33[a]	1.50±0.50[a]	26.12±4.45[a]
Acetyl salicylic acid	100 mg/kg	8.17±0.65[a]	1.67±0.21[b]	2.50±0.22[b]	2.33±0.21[a]	1.33±0.21[a]	0.00±0.00[a]	16.00±1.50[a]

[a]: $P<0.01$, [b]: $P<0.05$, compared with the normal saline group.

4. Discussion

The major folkloric uses of *Panicum maximum* have been in the treatment of malaria, infections, rheumatism pain, inflammation and diabetes[1]. It has been used to treat malaria and other febrille illnesses in Ibibios of Niger Delta region of Nigeria. These prompted the need to evaluate the antiplasmodial and analgesic potentials of the crude extract and fractions of the leaves of *Panicum maximum*.

In this work, LD_{50} was (480.00±4.91) mg/kg, and the extract was moderately safe.

The antiplasmodial properties of the extract and its fractions were investigated using standard models. It was found that both the extract and its fractions significantly reduced the parasitaemia in prophylactic, suppressive and curative models in a dose-dependent fashion. Some secondary metabolites of plants are said to have antiplasmodial activity. These metabolites include alkaloids, flavonoids and terpenoids[5,12,13]. These compounds, such as alkaloids, cardiac glycosides, tannins, saponins, terpenes and flavonoids have been reported to be present in this plant extract[1] and may in part contribute to the plasmocidal activity of this extract, which therefore may explain the mechanism of antiplasmodial effect of the extract and its fractions. However, elevation of red blood cell oxidation and inhibition of parasite's protein synthesis flavonoids and phenolic compounds in plants have been accountable to many antiplasmodial activities of plants[14]. These actions of plants antagonizes the oxidative destruction of erythrocytes by malaria parasite[15-17].

The extract significantly reduced acetic acid-induced writhing, formalin-induced hind paw licking and delayed the reaction time of animals (mice) to thermally induced pain. Acetic acid causes inflammatory pain by inducing capillary permeability[7,9], and in part through local peritoneal receptors from peritoneal fluid concentration of PGE_2 and PGF2α. It is used to distinguish between central and peripheral pain.

Formalin exhibits neurogenic and inflammatory pains[7] and measures both centrally and peripherally mediated activities that is characteristic of biphasic pain response. The first phase of formalin-induced hind paw licking is selective for centrally acting analgesics such as morphine[11]. The late phase of formalin-induced hind paw licking is peripherally mediated. Analgesic (nociceptive) receptors mediate both the neurogenic and non-neurogenic pains[7].

The study also shows that the extract significantly delays the reaction time of thermally-induced (hot plate) test. This model is selective for centrally acting analgesics and indicates narcotic involvement with opiod receptors[7].

The antinociceptive activities exerted by this extract may be attributed to the presence of secondary metabolites like saponins, flavonoids, tannins and terpenes. Flavonoids also have anti-inflammatory effects through its inhibition of the cyclo-oxygenase pathway[11]. That the extract inhibited neurogenic, non-neurogenic and narcotic pains may in part explain the mechanisms of its action and these effects are due to the components in the extract and fractions.

In conclusion, the results of this study support the ethnobotanical use of the plant in the treatment of febrile illnesses, malaria and pains. Further investigation is being advocated especially in elucidating cellular mechanisms and establishing structural components of the active ingredients with a view of standardizing them.

Conflict of interest statement

We declare that we have no conflict of interest.

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