Available online at *www.ujpronline.com*
Universal Journal of Pharmaceutical Research
An International Peer Reviewed Journal
ISSN: 2831-5235 (Print); 2456-8058 (Electronic)

RESEARCH ARTICLE

EFFECTS OF THE ESSENTIAL OIL OF DRIED FRUITS OF *PIPER GUINEENSE* (PIPERACEAE) ON NEUROLOGICAL SYNDROMES ASSOCIATED WITH CEREBRAL MALARIA IN MICE

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Article Info:

Article History:
Received: 5 December 2022
Reviewed: 9 January 2023
Accepted: 25 February 2023
Published: 15 March 2023

Cite this article:
OPOGGEN TS, DANIYAN MO, ASIYANBOLA ID, ADEOYE OB, Oyemitan IA. Effects of the essential oil of dried fruits of *Piper guineense* (Piperaceae) on neurological syndromes associated with cerebral malaria in mice. Universal Journal of Pharmaceutical Research 2023; 8(1):61-68.
https://doi.org/10.22270/ujpr.v8i1.902

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Abstract

Background: Cerebral Malaria (CM), is associated with neurological syndromes characterized by cognitive and neurobehavioral abnormalities. *Piper guineense* Schum and Thonnis known to possess anti-oxidant and central nervous system activities. In this study, effects of essential oil of *P. guineense* (EOPG) dried fruits on selected behavioral and functional indices in mice with cerebral malaria was evaluated.

Method: Mice with confirmed CM, following intraperitoneal inoculation with 1x10^7 *Plasmodium berghei* ANKA parasitized blood in 0.2 ml of normal saline, were randomly allocated into 14 groups (n=12), namely, parasitized control, quinine control, EOPG graded doses (6.25, 12.5, 25, 50, 100 and 150 mg/kg), and combination of quinine and EOPG graded doses. Quinine was administered at a dose of 20 mg/kg stat, then 10 mg/kg twice daily for next two days, while EOPG was administered once daily for 3 days, beginning from day 5 post inoculation. Non-parasitized (n=12) and parasitized controls were treated with the vehicle (5% Tween 80 in distilled water). Parasitemia, weight, survival and behavioral assessment using SHIRPA protocol were taken daily.

Results: EOPG showed anti-plasmodial activity in a dose dependent manner, significantly mitigated mortality rate at higher doses (100 and 150mg/kg), and exhibit dose dependent central nervous system protective effects. Also, except for quinine/6.25 mg/kg EOPG, 100% mortality was observed with combination groups, suggesting a potential to precipitate toxicity.

Conclusion: The study concluded that EOPG possesses antimalarial and central nervous system protective effects and may therefore serves to mitigate neurological syndrome in cerebral malaria.

Keywords: Cerebral malaria; Essential oil; *Piper guineense*; *Plasmodium falciparum;* SHIRPA.

INTRODUCTION

Cerebral malaria (CM), an important complication of severe *Plasmodium falciparum* malaria infection, is known to cause rapidly increasing deadly neurological disorders. It's responsible for high rate of mortality, especially among infected children in Sub-Saharan Africa each year[1,2], representing roughly 1% of all *P. falciparum* infections[3,4]. The aftermath effects of CM leaves survivors with acute or chronic physical handicap and neurological dysfunction, even after the infection has been treated[1,5]. Neuropsychiatric impairments can appear months or years after CM[6]. These manifestations vary between children and adults, and depend on the development of severe symptoms, such as coma and status epilepticus[6,7]. Though its etiology is exceedingly complex, and mechanical or vascular occlusion or sequestration, and inflammatory hypotheses, have been proposed, none could fully explain the pathogenesis of CM[1,8]. Detailed review on the roles of functional interactions of neurotransmitters and molecular chaperones in the pathology of cerebral malaria has been presented[9].To reduce mortality and increase quality of life, it is important to prevent potential neurological disorders that are often linger beyond antimalarial chemotherapy[5,10].To this end, adjuvants have been used in conjunction with very effective antimalarials for emergency situations and to lessen the risk of future occurrence[11-13].

Meanwhile, the use of medicinal plants for a variety of purposes has been unavoidable in all aspects of human survival, even as they continue to bring new therapeutic options[14-17]. *Piper guineense* Schum and Thonn (Piperaceae), popularly known as West African Black Pepper, is a perennial West African spice plant with over 700 varieties, grown for its aromatic and strong smell across the world's tropics[18,19]. Such biodiversity among one species of plants is a true reflection of its rigidity over the ages. With spicy taste, high mineral content, high fibers, as well as trace levels of carbohydrate, protein, and essential vitamins, *P. guineense* possesses high nutritional value, and its fruits and leaves have been used as condiments to flavor food in both domestic and commercial cuisines[18-21].The many biological and pharmacological activities of its various parts have been demonstrated[18,19]. These include antioxidant[22], anti-hyperglycemic effects[23], antinociceptive and anti-inflammatory activity[24], anti-plasmodial and analgesic properties[25], muscle relaxant properties[26], anti-convulsant[27], and hypothermic, sedative, and anti-psychotic activity[24,28], as well as displaying synergistic antibacterial and antifungal effects with *P. amarus*[29]. Specifically, the essential oil of *P. guineense* has been shown to have antioxidant properties, as well as antimicrobial[30], anti-inflammatory, antinociceptive, and central nervous system effects[24,28], possibly due to the abundance of its active phyto-constituents, including saponins, flavonoids, tannins, alkaloids, and phenols[31,32].

In this report, we investigated the potential benefits of the essential oil of *P. guineense* on cerebral malaria-induced neurological disorders. This was with a view to determining its potential usefulness as alternative pharmacotherapy for the management of neurological syndromes in CM. Our results provided evidence to support the antimalarial and CNS protective effects of the essential oil of *P. guineense* in a mouse model of cerebral malaria.

MATERIALS AND METHODS

Plant Materials and Hydro-distillation of Essential Oil

Dried fruits of *P. guineense* were purchased from the Central Market, Ondo Town, Ondo State between July and August, 2021. The fruits were identified and authenticated at the herbarium of the Faculty of Pharmacy, Obafemi Awolowo University (OAU), Ile-Ife, Osun State (FPI 2312). Further confirmation was done by checking the plant's name against an extensive medicinal plant databse (*http://www.theplantlist.org*). Using pestle and mortar, theair-dried fruits were mashed into coarse powders. The hydro-distillation of essential oil of *P. guineense* (EOPG) was carried out using a Clevenger-type apparatus as earlier described[24,33], yielding an average of 4.5 ml per 500 g. The amber glass bottle was used to store the pungent aromatic EOPG and kept in a freezer till use. The emulsification of the oil was done using Tween 80 to a final concentration of not more than 5%v/v prior to use.

Source and Care of Experimental Animals

Healthy Swiss mice of both sexes (18-22 g) were bred in the animal house of the Department of Pharmacology, Faculty of Pharmacy, OAU, Ile-Ife, Osun State, and housed in plastic cages with soft wood shavings as bedding. The animals were given free access to normal laboratory feed (Top feed grower, Premier feed mills co. LTD) and water *ad libitum*, and allowed at least 72 hours to acclimatize within the laboratory environment prior to use. All animal studies were carried out in accordance with the standards for the humane use and care of laboratory animals[34,35]. The study was approved by the Animal Health Research Ethics Committee, Institute of Public Health, OAU, Ile-Ife, Osun State, Nigeria (IPH/OAU/12/1782).

Median Lethal Dose (LD_{50}) and Working Doses Determination

The median lethal dose **(**LD_{50}) in mice was determined using the Lorke's method[36] with modifications. In the first phase, three increasing doses (10, 100 and 1000 mg/kg of EOPG) was administered intraperitoneally in three groups of nine mice (n=3). Following confirmation of total mortality at 1000 mg/kg, the second phase was conducted using 200, 300, 400, 500, 600 and 800 mg/kg doses with one mouse per dose. During both phases, mice were observed for signs of toxicity and mortality within the 24 hours of treatment. A preliminary assessment to determine safe working doses for this study was conducted using five doses (50, 100, 150, 200 and 250 mg/kg) below the LD_{50}. Randomly allocated mice (one per dose) were treated with single daily doses of the EOPG for 72 hours via the intraperitoneal route. Mice were daily monitored for signs of toxicity (hyperactivity, ataxia, muscle rigidity) and mortality.

Parasite inoculation and cerebral malaria development

The quinine sensitive strain of rodent experimental parasite, *Plasmodium berghei* ANKA (PbA), was obtained from the Institute for Advanced Medical Research and Training (IMRAT), University College Hospital, Ibadan, Oyo State, Nigeria, into donor mice, which were monitored till parasitized level of about 15 to 20%. Donor mouse was thereafter humanely euthanized by cervical dislocation and blood was obtained by cardiac puncture and diluted with normal saline to concentration of 1 x 10^7 parasitized red blood cells per 0.2 ml suspension as earlier described[37-39]. Each mouse was inoculated intraperitoneally with 0.2 ml of the inoculum suspension on Day 0 and the development of CM and onset of infection were monitored using Smith-Kline Beecham, Harwell, Imperial College, Royal London Hospital, phenotype assessment (SHIRPA) scales[40,41] as modified by Martins *et al.*,[42] and Wilson *et al.*,[43]. The onset of infection was determined as the point the CM is considered to have been fully developed using SHIRPA protocol, and beyond which the survival of the animal cannot be guaranteed without therapeutic intervention.

Experimental Design

Mice with confirmed CM were randomly allocated to the following groups (n=12): parasitized control (5% Tween 80 in distilled water), quinine (20 mg/kg stat, then 10 mg/kg twice daily), EOPG (6.25, 12.5, 25, 50, 100 and 150 mg/kg), and combination of Quinine and each of the dose of EOPG. Non-parasitized control (n= 12) was also treated with 5% Tween 80 in distilled water. Treatment was administered for 3 days starting from day 5 post-infection as earlier reported[38]. Parasitemia was determined daily and average percentage parasitemia was calculated as earlier described[37]. Also, body weight of each mouse was taken daily and relative body weight was determined as percentage of the weight on Day 0. In addition, parasite-induced mortality was recorded and used to evaluate the survival rate per treatment group.

Behavioral and Functional Evaluation

The SHIRPA protocol[40,41], as modified by Martins *et al.*,[42] and Wilson *et al.*,[43] was used in assessing behavioral and functional analysis. This study employed the SHIRPA behavioral battery of 25 semi-quantitative tests for reflex and sensory functions (corneal reflex, pinna reflex, righting reflex, toe pinch, and visual placing), neuropsychiatric functions (positional passivity, transfer arousal, spontaneous activity, and touch escape), spinocerebellar functions (body tone, grip strength), muscle and lower motor neuron functions (body position, tremor, gait,limb grasping, pelvic elevation, tail elevation, trunk curl, and wire maneuver), and autonomic functions (lacrimation, palpebral closure, piloerection, respiratory rate, skin color, and temperature), in all groups beginning from day 3 post infection. In this study, the scoring of the following activities was reversed: gait, lacrimation, tremor, palpebral closure, piloerection, positional passivity, righting reflex, and wire maneuver, similar to earlier report[42]. Details of the functional categories and their associated parameters, as well as the procedures for scoring are as earlier reported[40,42].

Statistical Analysis

Functional analysis was performed by quantitatively determining the contribution of each of the SHIRPA activities to their respective functions. Score for each behavioral activity per surviving animal per day was normalized by dividing with the highest score possible for that activity. Average of normalized scores per animal per day for all activities making up a functional category was then computed. This gives the functional score/value per animal per day. The mean of functional value per group of surviving animals per day was then calculated. Quantitative data were expressed as mean ± standard error of mean (mean ± SEM), mean (standard deviation {SD}) or median (lower/upper quarter) and analyzed using the one-way analysis of variance (ANOVA) followed by Turkey's posthoc test for parametric data or the Kruskal-Wallis test of multiple comparison with false discovery rate (FDR) for nonparametric data. All analysis were compared with unparasitized, parasitized and quinine controls using Graph Pad Prism version 8/9 (GraphPad, La Jolla, CA) with significant level set to $p<0.05$.

Table 1: Relative weight change following treatment with EOPG.

Treatment Groups	Day 0* (12)	Day 3 (12)	Day 4 (12)	Day 5 (12)	Day 6	Day 7	Day 8
Unparasitized Control	100±0.00	108.47±0.73	110.76±1.59	114.87±1.32	113.36±1.05 (12)	115.40±0.36 (12)	114.81±1.52 (12)
Parasitized Control	100±0.00	106.50±1.70	113.78±2.59	110.50±1.94	107.02±1.69^{a} (5)		
Quinine	100±0.00	107.34±1.61	110.45±2.24	108.65±1.76	106.22±1.04^{a} (8)	103.17±0.13^{a} (6)	99.28±1.22^{a} (5)
6.25 mg/kg	100±0.00	104.16±2.40	109.61±1.49	106.09±0.62^{a}	104.68±1.05^{a} (8)	97.00±0.07$^{a, c}$ (3)	96.63±0.23^{a} (3)
12.5 mg/kg	100±0.00	107.99±1.40	114.96±3.44	106.95±1.65^{a}	94.95±1.72$^{a, b, c}$ (8)	89.72±0.32$^{a, c}$ (5)	86.43±0.25$^{a, c}$ (3)
25 mg/kg	100±0.00	109.54±1.60	111.44±2.43	106.81±1.01^{a}	95.70±1.00 $^{a, b, c}$ (8)	91.04±0.75$^{a, c}$ (5)	93.92±1.18^{a} (3)
50 mg/kg	100±0.00	108.17±1.16	114.34±2.88	110.66±1.67	116.41±2.63^{c} (8)	115.31±1.13^{c} (5)	116.34±0.10^{c} (5)
100 mg/kg	100±0.00	107.73±2.64	117.98±2.58	114.25±1.13	117.79±2.85$^{b, c}$ (8)	122.64±3.02$^{a, c}$ (8)	120.58±0.78$^{a, c}$ (6)
150 mg/kg	100±0.00	105.71±1.40	118.96±2.40	115.39±2.64	118.69±1.77$^{b, c}$ (11)	121.76±2.22$^{a, c}$ (8)	119.24±1.01$^{a, c}$ (8)
Quinine + 6.25 mg/kg	100±0.00	109.04±1.31	116.93±2.36	113.32±2.42	109.63±1.45 (5)	100.73±0.66$^{a, c}$ (3)	96.48±1.04^{a} (3)
Quinine + 12.5 mg/kg	100±0.00	107.43±2.52	110.42±1.27	98.33±1.41^{a,b,c}			
Quinine + 25 mg/kg	100±0.00	106.22±1.51	114.91±1.67	93.43±0.96^{a,b,c}			
Quinine + 50 mg/kg	100±0.00	107.07±0.62	115.35±2.78	92.77±0.80^{a,b,c}			
Quinine + 100 mg/kg	100±0.00	108.86±2.25	115.40±1.75	93.99±1.57^{a,b,c}			
Quinine + 150 mg/kg	100±0.00	106.39±1.22	117.39±3.69	98.20±1.26^{a,b,c}			

***** Significantly different when compared with Days 3, 4, 5, 6, 7 and 8; ^{a}Significantly different when compared with the unparasitized control group; ^{b}Significantly different when compared with the parasitized untreated control group; ^{c}Significantly different when compared with standard (Quinine) control group; Results expressed as Mean±SEM and values with $p< 0.05$ are considered significant; Number of surviving mice are as indicated in parenthesis

RESULTS

Determination of Median Lethal Dose (LD_{50}) and Working Doses

The median lethal dose (LD_{50}) of EOPG via the intraperitoneal route (i.p) in mice was found to be 273.86 mg/kg, suggesting that the oil is relatively toxic. Therefore, to prevent death arising from the potential toxicity of the test agent following repeated dosing, we conducted a preliminary assessment of the effects of selected doses of EOPG on unparasitized mice, following once daily intraperitoneal administration for three consecutive days. The result showed that doses ≤150 mg/kg body weight can be considered as safe working dose.

Effects of EOPG on Weight, Parasitemia and Survival

Effects of EOPG on Weights

The relative body weight changes (Table 1) revealed significant ($p<0.05$) increase in weights across all treatment groups prior to dosage administration when compared to Day 0.

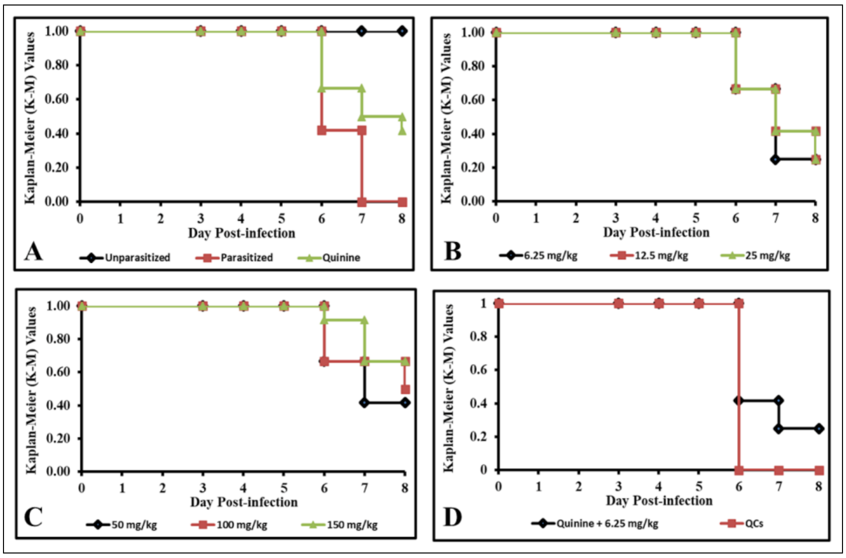

Figure 1: Kaplan-Meier's survival plot following treatment of CM mice with varying doses of EOPG. Control groups (A); Varying doses of EOPG (B and C); Quinine/EOPG combinations (D). QCs in panel D represents the survival plot of all the combinations that died within 24 hours following the first administration.

These increases dropped sharply on day 5 following the establishment of CM in parasitized mice. Following the dosage administration, the downward trend continued in most of the parasitized groups from Days 6 to 8, except at 50, 100 and 150 mg/kg body weight of EOPG which showed significant increases in weights, suggesting that at higher doses the oil may possess potential to arrest or mitigate loss of weight that often rapidly characterize malaria infections. However, except for combination of quinine and 6.25 mg/kg, all animals in other combinations died within 24 hours of first dosage administration (Table 1), suggesting possible potentiation of toxicity. Also, the quinine/6.25 mg/kg combination did not confer any advantage over either used alone.

Effect of EOPG on the level of Parasitemia

The onset of CM infection, following intraperitoneal inoculation of 1 x 10^7 parasitized red blood cells was determined to be Day 5 post-inoculation using SHIRPA protocol. Beyond Day 5, the survival of the animals cannot be guaranteed without therapeutic intervention. Therefore, treatment began on Day 5 and serves as reference point to monitor the disease progression and the effects of therapeutic intervention. All animals across treatment groups had increases in parasitemia level from day 3 to 5 post inoculation (before test agents administration), followed by mostly significant reduction after treatment as compared to the parasitized control (Table 2). Specifically, while there are consistent increases in the level of parasitemia with 6.25 mg/kg EOPG, similar to parasitized control, the administration of 12.5 – 150 mg/kg EOPG showed significant decreases in parasite load in a dose dependent manner, when compared to parasitized control at the onset of CM infections (Day 5). Also, mostly significant reductions in parasitemia level were observed daily with repeated dosing for each dose level. Moreover, the abilities of 50, 100 and 150 mg/kg EOPG to reduce parasitemia were comparable to the standard control (Quinine). However, the combination of quinine with 6.25 mg/kg EOPG caused a significant reduction in the ability of quinine or EOPG to reduce parasitaemia, suggesting a potential for antagonistic interaction.

Effects of EOPG on the survival rate of the animals

The Kaplan-Meier survival plot (Figure 1) revealed that a significant percentage of the animals in most of the treated groups survived the infection longer following the 72-hour treatment after the development of experimental cerebral malaria (ECM) when compared with parasitized control group. The 6.25, 12.5, and 25 mg/kg of EOPG (Figure 1B), and quinine/6.25 mg/kg EOPG combination (Figure 1D) showed comparable ability to increase survival rate in CM mice when compared to parasitized untreated, but at different rate and significantly less than quinine (Figure 1A). However, while 50 mg/kg EOPG showed a comparable survival rate with quinine, 100 and 150 mg/kg EOPG (Figure 1C) showed an improved ability to preserve the animal better, having relatively higher survival rate when compared to the standard.

Effects of EOPG on behavioral assessments

The primary screen of the protocol aids the quantitative assessment of several parameters thereby providing a measure with which phenotypic expressions in mouse are scored to enable comparison of results. Results obtained for Days 5 to 8, from the SHIRPA behavioural battery of the 25 semi-quantitative tests, assessed for motor and lower neuron, spinocerebellar, reflex and sensory, neuropsychiatric and autonomic functions, following daily administration of the test agents, are as shown in Supplementary Tables 1 to 4 (Tables S1–S4). On Day 5, the assessed indices largely showed significant reduction in scores when compared to unparasitized control, confirming CM development and onset of CM infection. As treatment progresses, gradual and dose dependent improvement in activities with repeated dosing of test agents can be observed.

Table 2: Percentage parasitemia following daily administration of EOPG.

Treatment Groups	% Parasitemia of Surviving Mice Per Day					
	Day 3 (12)	Day 4 (12)	Day 5 (12)	Day 6*	Day 7*	Day 8*
Parasitized Control	5.07±0.11	6.29±0.18	14.37±0.13	17.78±0.02 (5)		
Quinine	5.11±0.31	6.01±0.22	13.91±0.19	6.88±0.14^{a} (8)	4.73±0.03 (6)	2.50±0.09 (5)
6.25 mg/kg	5.15±0.11	6.51±0.15	13.81±0.10	16.90±0.15$^{a, b}$ (8)	28.79±0.11^{b} (3)	
12.5 mg/kg	4.93±0.08	6.52±0.13	13.92±0.07	15.69±0.04$^{a, b}$ (8)	13.99±0.94^{b} (5)	8.51±0.30^{b} (3)
25 mg/kg	4.86±0.06	6.23±0.07	14.01±0.10	14.30±0.59$^{a, b}$ (8)	13.42±0.10^{b} (5)	12.14±0.10^{b} (3)
50 mg/kg	4.78±0.25	6.17±0.16	14.82±0.10	11.53±0.10$^{a, b}$ (8)	3.32±0.05^{b} (5)	4.35±0.32^{b} (5)
100 mg/kg	4.54±0.16	6.18±0.25	14.27±0.29	8.31±0.18$^{a, b}$ (8)	6.20±0.04^{b} (8)	5.53±0.11^{b} (6)
150 mg/kg	4.78±0.15	6.02±0.15	13.66±0.05	7.20±0.14^{a} (11)	4.88±0.34 (8)	3.33±0.41^{b} (8)
Quinine + 6.25 mg/kg	5.29±0.16	6.10±0.05	13.62±0.13	8.13±0.04$^{a, b}$ (5)	9.47±0.09^{b} (3)	9.84±0.10^{b} (3)

Data are expressed as Mean±Standard Error of Mean (SEM) and values are considered significant at $p < 0.05$; *All values are significantly different from parasitized control at the onset of infections (Day 5); ^{a}Significantly different when compared to parasitized control on same day; ^{b}Significantly different when compared to quinine control on same day; D3 to D8 are Days 3 to 8 respectively, and number of surviving mice are as indicated in parenthesis

Table 3: SHIRPA functional analysis following treatment with EOPG.

	Muscle & lower motor neuron function		Spinocerebellar function		Reflex/Sensory function		Neuropsychiatric function		Autonomic function	
	Day 5	Post-Treatment	Day 5	Post-Treatment	Day 5	Post-Treatment	Day 5	Post-Treatment	Day 5	Post-Treatment
Unparasitized	0.998±0.017*	0.993±0.002	1.000±0.000*	1.000±0.000	1.000±0.000*	1.000±0.000*	0.987±0.016*	0.991±0.001*	0.995±0.012*	0.995±0.000
Parasitized Control ^{R}	0.690±0.018		0.759±0.016		0.829±0.023^{z}		0.815±0.015		0.775±0.019	
Quinine	0.672±0.021$^{\#}$	0.949±0.012^{xy}	0.737±0.025$^{\#}$	0.931±0.037^{y}	0.769±0.017$^{\#y}$	0.981±0.010*	0.785±0.037	0.824±0.015^{y}	0.789±0.021	0.744±0.032^{x}
6.25 mg/kg	0.721±0.071	0.851±0.012^{xyz}	0.775±0.035	0.889±0.056	0.860±0.067	0.694±0.047	0.825±0.012$^{\#}$	0.905±0.020y^{z}	0.753±0.028$^{\#}$	0.870±0.019^{xyz}
12.5 mg.kg	0.720±0.064	0.828±0.021^{xyz}	0.738±0.029$^{\#}$	0.917±0.024^{xy}	0.817±0.059	0.784±0.029	0.802±0.017$^{\#}$	0.905±0.011y^{z}	0.739±0.038	0.828±0.020^{x}
25 mg/kg	0.676±0.019$^{\#}$	0.818±0.029^{xyz}	0.725±0.016$^{\#}$	0.888±0.015^{xy}	0.727±0.013$^{\#yz}$	0.835±0.012	0.824±0.050	0.913±0.010y^{z}	0.776±0.030	0.877±0.040
50 mg/kg	0.719±0.036$^{\#}$	0.872±0.018^{xyz}	0.765±0.012	0.826±0.032^{x}	0.750±0.024$^{\#}$	0.886±0.007	0.788±0.065	0.885±0.005y^{z}	0.808±0.016$^{\#}$	0.913±0.040^{yz}
100 mg/kg	0.641±0.046$^{\#}$	0.883±0.062^{y}	0.783±0.029$^{\#}$	0.921±0.029^{y}	0.804±0.019$^{\#}$	0.957±0.001^{y}	0.812±0.024$^{\#}$	0.903±0.015y^{z}	0.797±0.006$^{\#}$	0.917±0.058^{yz}
150 mg/kg	0.713±0.016$^{\#}$	0.970±0.011^{y}	0.772±0.017$^{\#}$	0.959±0.011^{xy}	0.850±0.017$^{\#z}$	0.956±0.016^{y}	0.817±0.026$^{\#}$	0.928±0.017y^{z}	0.798±0.006$^{\#}$	0.977±0.011^{yz}
Quinine + 6.25 mg/kg	0.705±0.018	0.673±0.076^{xz}	0.747±0.018$^{\#}$	0.938±0.062^{y}	0.822±0.011^{z}	0.807±0.046	0.778±0.064	0.888±0.027	0.758±0.032	0.792±0.007^{x}

Day 5 is as contained in Suppl. Table 5, and Post-infection is the Mean±SEM for values on Days 6, 7, and 8 of Suppl. Table 5 $^{*, \#, x, y, z}$indicates significant difference when compared with other groups, Post-treatment, Unparasitized control, Parasitized control on Day 5, and Quininestandard control respectively. Significant value was set at $p<0.05$. ^{R} No Post-treatment values for Parasitized control as none of the animals survived beyond Day 6 post inoculation.

Essentially on Days 7 and 8, most activities appeared to be completely reversed at higher doses of 100 and 150 mg/kg, comparable to unparasitized control (Tables S3 and S4). Interestingly, Quinine/6.25 mg/kg EOPG showed comparable improvement in SHIRPA activities, suggesting potential for synergistic activity with Quinine at lower dose. Moreover, observed improvements or reduced deterioration rate in the single higher doses of the extract (50, 100, 150mg/kg) as well as the lowest combined dose (Quinine + 6.25 mg/kg), were significantly higher than parasitized control. Meanwhile, lacrimation, trunk curl, limb grasping, and righting reflex were not altered in the presence or absence of the disease, with or without treatment, throughout the study period. Similarly, SHIRPA functional analysis showed comparable trend (Table S5). By Day 8, all functions that were assessed have been restored to normal level by quinine, as well as 100 and 150 mg/kg EOPG, similar to unparasitized control. However, while significantly improving spinocerebellar and autonomic functions, quinine/6.25 mg/kg significantly caused a reduction in muscle and lower motor neuron function, as well as reflex and sensory function, compared to quinine alone, but produced similar effects on neuropsychiatric function (Table 3).

DISCUSSION

Malaria infection remains catastrophic with increasing cases of resistance to known treatment modalities, and while severe infections and neurological complications from CM seems to be declining globally, the burden is now skewed towards Africa region accounting for about 51% of all global malaria cases[4]. With increasing resistance to available and affordable drugs, the need for continuous search for alternative pharmacotherapy is imperative. In addition, the persistence of the disease and changes in resistance mechanism with time, generate the need for a radical solution. Essentially, the need to arrest the fatal neurological complications, which often linger to adulthood, is leading the way to more intensive research, with particular focus on natural products, in order to uncover new and effective pharmacotherapeutic agents. In this study, *P. guineense*, a plant with reported anti-plasmodial and CNS activities[25,28], was investigated for its potential benefits in mitigating the neurological symptoms associated with CM.

Obtained results confirmed the anti-plasmodial potential of *P. guineense*, as previously reported[25]. However, to our knowledge, this is the first report of the antiplasmodial effects of the essential oil derived from any part of the plant. The anti-plasmodial effects of EOPG were dose-dependent, significantly reducing the level of parasitaemia with increasing doses, and with higher doses (100 and 150 mg/kg) being statistically comparable (p<0.05) to the standard (Quinine) control group. In addition, EOPG showed potential to arrest weight loss often associated with malaria, and increase survival rates in animals.

Meanwhile, the brain's irreplaceable functions, distinctive structure, and incidental aberration can be used to infer functional activities in both normal and diseased states. The SHIRPA protocol main screen provides a reproducible quantitative observational assessment of functional profiles that can help to describe functional anomalies, allowing proper analysis of associated physiologic and pathophysiologic conditions[40,41].

In this study, the expected onset of neurological problems in all parasitized mice was confirmed by the daily decline in frequency and intensity of functional activities from days 3 to 5. By day 5, all behavioral activities related to neuropsychiatric and motor functions, as well as spinocerebellar and sensory functions, and autonomic functions, that served as indicators of neurological functions, had been clearly established across all groups[42]. It should be noted, that in CM-induced mice with impaired muscle and lower motor neuronal function, parasite clearance alone does not imply that further progression of neurological syndromes is slowed as long as the blood brain barrier (BBB) is compromised[44]. Also, it has been noted that cognitive impairment may be considered to revolve around the activities of inflammatory cytokines and BBB vascular permeability to induce a deformity in the memory system due to altered memory caused by sequestered inflammatory cells in the brain vasculature[45]. While the onset of neurological syndrome in humans may not necessarily imply mortality because there may be recovery after paroxysm[1,8,10], in experimental CM, the probability of losing a significant number of animals hours after CM sets in is high[42,46]. Interestingly, a large number of animals given doses of EOPG alone survived longer, implying that EOPG has the potential to reduce mortality. Also, it's worth noting that EOPG not only demonstrated capacity to reduce the level of parasitaemia and increase survival rate, but also showed capacity to dose-dependently arrest the progression of neurological and functional aberrations, and at higher doses, completely restored functional activities to normal. These effects may be linked to the reported CNS protective and anti-inflammatory activities of EOPG[24,28].

Meanwhile, since parasitemia eradication does not prevent the development of NS once CM has set in, the need for adjuvant therapy to prevent or ameliorate cognitive deficits, has been demonstrated[44]. However, our attempt at combining EOPG with Qunine did not produce desired benefit, due to high rate of mortality. Majority of the animals given a combination of Quinine and graded doses of extract died within 24 hours of their first treatment, save Quinine/6.25mg/kg, raising concerns about the potential toxic effects of indiscriminate use of this plant within the larger population. With low LD_{50} of EOPG, and the potential toxicity of Quinine, the mortality linked to Quinine/EOPG combination may have resulted from possible potentiation of their individual inherent toxicity, and/or unfavourable pharmacological interaction. Therefore, we submit that the toxicologic potential of this combination need further investigation.

Limitations of the study

This study is limited to animal experimentation only and may not be directly translated to humans without further investigation. It is also limited by resources to unravel the mechanism(s) of the reported effects at cellular and molecular levels.

CONCLUSIONS

The findings from the present study suggest that, despite being slightly toxic, the oil has anti-plasmodial and CNS protecting properties in experimental CM. As a result, its potential use alone could help to alleviate NS associated with CM. However, while we seek further investigation into the potential toxicity of combining EOPG and Quinine, caution should be maintained in concurrent administration or indiscriminate use of EOPG with Quinine.

ACKNOWLEDGEMENTS

We appreciate Mr. I. I.Ogunlowo, Herbarium Officer, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun State, for his assistance in identification and authentication of the plant material used in this study.

AUTHOR'S CONTRIBUTION

OPOGGEN TS: Performed the experiments. **DANIYAN MO:** conceptualization and project administration. **ASIYANBOLA ID:** data analysis, drafting and review. **ADEOYE OB:** data analysis, initial draft of the manuscript. **Oyemitan IA:** methodology, investigation. All the authors approved the finished version of the manuscript.

DATA AVAILABILITY

The datasets generated during this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

None to declare.

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