Larvicidal and antiplasmodial studies of *Eucalyptus camaldulensis* (*Myrtaceae*) Leaf

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Abstract

Eucalyptus camaldulensis is a medicinal plant used as insect repellant and antimalarial agent in ethnomedicine. This study examined the antiplasmodial and larvicidal potential of *E. camaldulensis* leaf extract and also identified the plant extract's most active fraction(s). The acute oral toxicity test of the methanol extract was evaluated using Lorke's method. The larvicidal assay was performed on the extract and partitioned fractions according to the 2005 World Health Organization guidelines, while the 4-day chemosuppressive and curative antimalarial assays were carried out against *Plasmodium bergei bergei*. Endosulphan and chloroquine (10 mg/kg) was used as the positive controls for the larvicidal and antiplasmodial assays, respectively, while tween 80 in normal saline (1%) was the negative control. The methanol leaf extract of EC showed good larvicidal activity across all tested concentrations (LC_{50} 3.79 ± 0.64 mg/mL), while the aqueous fraction with LC_{50} of 2.80 ± 0.14 mg/mL was the most active. Its acute toxicity test showed it was safe up to 5000 mg/kg. The extract significantly increased dose-dependent antiplasmodial activity for the chemosuppressive and curative models ($p < 0.05$) than the negative control. At 800 mg/kg, EC gave chemosuppressive (53.69 ± 1.62%) and curative (81.26 ± 1.87%) activities, which was significantly lower than that of 10 mg/kg chloroquine (82.00 ± 0.57% and 92.51 ± 0.22% respectively). The aqueous partitioned fraction gave the highest chemosuppression (73.84 ± 2.73%) at 80 mg/kg, which was comparable to the positive control. The methanol extract of *E. camaldulensis* leaf is a promising larvicidal and antimalarial agent that could proffer a solution to vector control and the prevalence of malaria.

Keywords: Eucalyptus camaldulensis, larvicidal; Malaria; Chemosuppressive; Curative

Introduction

Parasitic diseases are life-threatening infectious diseases induced by parasites such as protozoa, worms, arthropods, Annelida and molluscs (Wang 2017). It is a global menace predominant in warm and humid areas of tropical, subtropical and temperate regions, especially in developing countries (Brugman et al. 2018). Many human parasites have animal reservoirs or are transmitted via insects. Malaria, yellow fever, dengue fever, schistosomiasis, filariasis and Japanese encephalitis are transmitted mainly by mosquitoes (Brugman et al. 2018). The extended accessibility of malaria determent techniques and strategies, including efficient vector control and the administration of prophylactic antimalarial medications, has significantly reduced the burden of this illness worldwide in the past 20 years (Tizifa et al. 2018). Due to its excellent efficacy in mitigating infection and reducing disease transmission, vector management is a important part of malaria check and eradication methods (Stone et al. 2016). Using larvivorous fish and larvicides, adequate drainage systems and other environmental controls can help prevent and control malaria (Wang 2017; Tizifa et al. 2018).

Larvicides act by eliminating mosquito larvae before they can develop into adults. Specific formulations are effective when they contact mosquito larvae, whereas others are active when mosquitoes consume them (Fiorenzano et al. 2017). The evolution of resistance to chemical-based larvicides has resulted in the increased hunt for larvicidal compounds from natural sources by most researchers, most of which are biodegradable, environmentally safe and pose no risk to humans or animals (Podder and Ghosh 2019; Chatterjee et al. 2023).

Malaria is an severe disease caused by the protozoan *Plasmodium*. It is transmitted through the bites of infected female *Anopheles*. Of the five major parasites that cause malaria in humans, *P. falciparum* and *P. vivax* are the most prevalent on the African continent (Twohig et al. 2019; Abdulraheem et al. 2022). *Plasmodium vivax* is a malaria parasite reported to be prevalent in most countries outside the shores of African (Abdulraheem et al. 2022). Fever, headache and chills are symptoms of malaria, which usually appear 10–15 days after the infective mosquito bite and may be mild and difficult to recognize as malaria (Hassan et al. 2022). If malaria infection caused by *P. falciparum* is left untreated, it often resulted to acute illness and sometimes to death within a day (Hassan et al. 2022).

The latest World malaria report shows 247 million cases of malaria in 2021 as opposed to 245 million cases in 2020, while the reported estimated deaths were 619 000 in 2021 and 625 000 in 2020 (World Health Organization (WHO) 2022). The WHO African Region said a disproportionately high degree of the global malaria burden (World Health Organization (WHO) 2022). In 2021, children (under 5 years old) accounted for about 80% of malaria deaths in Africa (World Health Organization (WHO) 2021). Nigeria, Democratic Republic of Congo, United Republic of Tanzania and Niger with 31.3, 12.6, 4.1 and 3.9% of malaria-related deaths, respectively, accounted for over half of this death globally (World Health Organization (WHO) 2022).

Medicinal plants produce many secondary metabolites that are sources of bioactive organic compounds that defend against an infection (Akinwunmi et al. 2022). These substances may work as attractants, insecticides, antifeedants, oviposition inhibitors, repellents, growth inhibitors, juvenile hormone analogues, and moulting hormones (Berestetskiy and Hu 2021). *Eucalyptus camaldulensis* (Myrtaceae) (EC), or Murray Red Gum, is a medicinal plant found in Nigeria, West African countries, Asia and Australia (Sebei et al. 2015). The plant treats microbial infection, insect repellant, wound healing, aches, respiratory diseases and malaria

(personal communication). The Igala people of Kogi State traditionally use the leaf in Nigeria to treat malaria and fever (Ghareeb et al. 2018; Ayalew et al. 2022). The anti-inflammatory, analgesic, anti-nociceptive, cytotoxic, anti-parasitic, insecticidal and antidiabetic activities of different morphological parts of *E. camaldulensis* have been reported (Ganesh et al. 2012; González-Burgos et al. 2018; Aljawdah et al. 2022). The larvicidal activity of the plant's essential oil and leaf extracts has been earlier studied (Idris et al. 2008; Cheng et al. 2009; Medhi et al. 2010). The antimalarial activity of the chloroform and aqueous extract of the leaf extract of EC has been assessed by Ishaya et al. (Ishaya et al. 2019) and Anigboro et al. (Anigboro et al. 2020), respectively. Also, different classes of phytochemicals like polyphenols, terpenoids and essential oils, responsible for larvicidal and antimalarial activity has been isolated from EC extracts (Ghareeb et al. 2018; Cheng et al. 2009; Medhi et al. 2010; Anigboro et al. 2020). However, none of these studies identified the fraction responsible for the activities elicited by the extract. Therefore, this study assessed the antiplasmodial and larvicidal activities of the methanol extract and the partitioned fractions of EC leaf to identify the most active fraction(s).

Materials and methods

Plant collection, extraction and partitioning

The leaf of EC was collected within Obafemi Awolowo University (OAU) campus. Mr I.I. Ogunlowo identified the plant, and the authenticated specimen was deposited at the Faculty of Pharmacy Herbarium, Department of Pharmacognosy, OAU, Ile-Ife, with voucher number FPI 2437. The leaves were dried openly and pulverized. A 500 g of the dried powder was macerated in methanol (1500 mL) for 72 h and shake intermittently. The resultant extract was filtered, evaporated to dryness using a rotary evaporator (SE-100N, Shimadzu), weighed and stored. A 70.0 g of the extract was placed in a beaker and retaken into distilled water (500 mL) and partitioned consecutively into n-hexane and dichloromethane to obtain their n-hexane (16 g), dichloromethane (29 g) and aqueous (18 g) fractions.

Larvicidal activity of extract and fractions

The methanol extract and partitioned fractions were assayed for larvicidal activity using the World Health Organization 2005 guidelines with slight modifications (Adebajo et al. 2014). The extract and partitioned fractions were individually dissolved in Tween 80 (0.2%, v/v). Healthy fourth instar *Culex quinquefasciatus* larvae (Anigboro et al. 2020) were released into each assay cup. Each concentration was replicated five times. Endosulphan, a commercial insecticide, was used as the positive and Tween 80 in distilled water as negative controls. After that, the number of survived larvae was counted after 24 and 48 h of exposure. The average percentage mortality was calculated, and these were used to determine the LC_{50} and LC_{90} values (Famuyiwa and Kolawole 2019).

Animals and parasite

Mice of either sex (18–24 g) were obtained from the Animal House of College of Health Sciences, OAU, where they were put in aluminum cages with wood shavings used as beddings and allowed free access to water and food (Growers' mash) under 12 h day/ night cycle. They were acclimatized for a week before use. *Plasmodium berghei berghei* strain NK65 (Chloroquine sensitive) was obtained from the Institute of Advanced Medical Research and Training (IMRAT), University College Hospital, Ibadan. The parasite strain was preserved via

serial passage of blood taken from an infected mouse into an uninfected mouse. The Board of Postgraduate College, OAU, approved the animal experimental methodology with the Registration Number SCP17/18/H/1272. The animals were handled following the National Institutes of Health (NIH) Guide for the Care and Use of laboratory animals; (NIH Publication, No. 83-123 (revised), 1985) was followed in the handling caring of the mice.

Ld_{50} determination

Lorke's method was used to determine the lethal dose of the extract. Twelve mice were divided into two phases: Phase 1 involved nine mice divided into three groups of three mice each. Each group of mice was administered 10, 100 and 1000 mg/kg of the extract and observed for 24 h to monitor their behavioural responses as well as mortality (no mortality in Phase 1 would enable the experiment to proceed to Phase 2. Otherwise, the LD_{50} will be decided at Phase 1). Phase 2 involved using three mice, which were grouped into three of one mouse each. The mice were administered higher doses (1600, 2900 and 5000 mg/kg) of the extract and then observed for 24 h for behaviour and mortality. No mortality at this stage confirmed the extract as being non-toxic (Lorke 1983).

The formula for LD_{50} calculation: $LD_{50} = \sqrt{(D_0 \times D_{100})}$.

D_0 = Highest dose that gave no mortality.

D_{100} = Lowest dose that produced mortality.

In vivo antimalarial assays

Parasite inoculation

The donor mouse was *anaesthetized,* sacrificed, and blood was withdrawn through cardiac puncture into a heparinized bottle to prepare the inoculum. It was diluted with normal saline so that 0.2 mL of the inoculum would contain 1.0×10^7 parasitized red blood cells. The dilution factor was obtained by calculating each mouse's inoculum size and volume. Each test mouse was inoculated with 0.2 mL of the inoculum intraperitoneally (Aladesanmi et al. 2022).

Chemosuppressive test

The chemosuppressive activity of the methanol extract and chloroquine, 10 mg/kg (the standard drug), was evaluated against early infection (Ryley and Peters 1970). The inoculated mice were randomly divided into 6 groups (I–VI) of five mice each. Two hours after infection, 0.2 mL of the extract (100, 200, 400 and 800 mg/kg) were given orally using an oral cannula to group I-IV. Chloroquine 10 mg/kg was administered to group V (positive control), and normal saline was given to group VI (negative control). The administration procedure was repeated for 3 consecutive days. The parasitaemia level was determined for each mouse on day 4 (D4) by withdrawing a thin blood smear from their tail on a slide, fixed with methanol, stained with Giemsa and viewed under the microscope by cell counting of 5 fields (Ryley and Peters 1970; Tona et al. 2001). The mean parasitaemia in each group of 5 mice was determined to get the percentage of chemosuppressive activities of the extract and fractions using the formula:

$$\% \text{ Chemosuppression} = \frac{(\text{PN} - \text{PT})}{\text{PN}} \text{ X } 100$$

PN and PT are the mean parasitaemia in the negative control and the test groups, respectively (Adesida et al. 2021).

Curative test model (Ryley and Peters, 1970)

Thirty mice inoculated with *P. berghei* were separated at random into 6 groups (I–VI). Each group has five mice each. Seventy-two hours after inoculation, mice in Groups I–IV were administered with the methanol extract as described in the suppressive test model. The same doses were repeated daily for four consecutive days (D_1–D_4). Preparation of blood smears was done daily to assess the parasitaemia levels for 5 days (D_0–D_4). The % clearance and the average survival time were also determined.

Mean survival times

The mice were observed for 28 days for time of death. The survival time for each mouse was noted as days, and the mean survival time for each group was calculated as days ± SEM (Aladesanmi et al. 2022; Adesida et al. 2021).

Median effective doses ED_{50} and ED_{90}

A graph of the test doses in mg/kg against chemosuppression in percentage was automatically plotted using Microsoft Excel 2007 from which the median effective doses ED_{50} and ED_{90}, the doses that would give 50 and 90% chemosuppression, were forecast and recorded as mg/kg ± SEM.

Statistical analysis

Values were expressed as mean ± SEM and analyzed using Microsoft Excel 2010. One-way Analysis of Variance (ANOVA) was used to determine the statistical significance. This was followed by posthoc test of Student Newmann Keul to compare in order to determine the origin of significant difference for every value. Value of $P < 0.05$ was significant statistically.

Results and discussion

Larvicidal activity of the extract

The larvicidal property of EC was validated by evaluating its activity against *C. quinquefasciatus* larvae at 24 and 48 h. The results of the susceptibility of *C. quinquefasciatus* larvae to EC at exposure times of 24 and 48 h, respectively, are shown in Table 1.

Table 1. Larvicidal activities of EC against *C. quinquefasciatus*

Extract/drug	24		48 h		Percentage Mortality (%)
	h LC_{50}	LC_{90}	LC_{50}	LC_{90}	
EC	4.84 ± 0.58^{b}	7.69 ± 1.33^{b}	2.85 ± 0.17^{b}	4.42 ± 0.66^{b}	56
ED	1.03 ± 0.10^{a}	1.21 ± 0.18^{a}	0.85 ± 0.13^{a}	1.73 ± 0.23^{a}	83

Keys: Doses in mg/mL; One-way analysis of variance followed by the Student–Newman–Keuls test revealed a significant difference between values within columns with different superscripts ($p < 0.05$). EC methanol extract of E. camaldulensis leaf, ED Endosulphan

The obtained results showed that EC induced good activity (LC_{50}/LC_{90} 2.85 0.17, 4.42 0.66 mg/mL) at 48 h and moderate activity (LC_{50}, LC_{90} 4.84 0.58, 7.69 1.33 mg/mL) at 24 h. The extract's larvicidal efficacy was time-dependent, as potency markedly increased with longer exposure times. The commercial organochlorine insecticide Endosulphan, which was utilized as the positive control, was, however, noticeably ($p < 0.05$) more effective than EC leaf extract. Furthermore, the EC methanol extract gave percentage mortality of 56% against *C. quinquefasciatus.* The result obtained further support the percentage mortality (60%) previously reported against by Idris et al. (2008). Additionally, the activity of EC extract was not comparable to endosulphan at 24 and 48 exposure times. The larvicidal activity of this extract against Aedes was comparable to that of *Kalanchoe crenata* leaf, *Mimosa pudica* whole plant, *Nauclea latifolia* root, and *Pentaclethra macrophylla* extracts (Kamaraj et al. 2010).

Larvicidal activity of partitioned fractions

Further fractionation of the methanol extract using solvents with increasing polarity was done to look at potential improvements in larvicidal activity. The outcomes demonstrated that during the test period, the aqueous fraction (AQ) triggered the highest larvicidal efficacy (Table 2).

Table 2. Larvicidal activities of the partitioned fractions of EC against *C. quinquefasciatus*

Extract/drug	24 h		48 h	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}
N-HEX	4.42 ± 0.66^{b}	6.84 ± 1.52^{b}	3.67 ± 0.13^{b}	6.67 ± 0.25^{b}
DCM	4.56 ± 0.56^{b}	7.82 ± 1.31^{b}	4.13 ± 0.16^{b}	7.44 ± 0.23^{b}
AQ	5.92 ± 0.51^{c}	9.96 ± 0.94^{b}	2.80 ± 0.14^{c}	4.27 ± 0.37^{c}
ED	1.03 ± 0.10^{a}	1.21 ± 0.18^{a}	$0.85 \pm 0.13a$	1.73 ± 0.23^{a}

Keys: Doses in mg/mL; One-way analysis of variance followed by the Student–Newman–Keuls test revealed a significant difference between values within columns with different superscripts ($p < 0.05$). *N-HEX* n-hexane fraction, *DCM* Dichloromethane fraction, *AQ* Aqueous fraction; ED Endosulphan

According to Table 2, the solvent fractions' larvicidal efficacy increases from 24 to 48 h. Both the *N-HEX* and *DCM* fractions of EC elicited a moderate to good larvicidal activity at 24 and 48 h, with no discernible difference ($p < 0.05$) between them. After 48 h of treatment, the *AQ*

fraction had the greatest larvicidal efficacy (LC50, LC90: 2.80 0.14, 4.27 0.37 mg/mL) against *C. quinquefasciatus* larvae (Table 2). The larvicidal activity of the solvent fractions and Endosulphan (positive control) differed significantly. As a result, the activity order of the partitioned fractions is water > n-hexane > dichloromethane. The outcomes of this investigation supported earlier discoveries that of EC.

LD_{50} determination

The lethal dose (LD50) of EC was greater than 5000 mg/kg, indicating that it may not be toxic and is safe. In ethnomedicine, it is openly utilized for the treatment of malaria.

Antiplasmodial tests of EC

Chemosuppressive (4-day) test

In this investigation, the four-day in vivo chemosuppressive test was used to assess the antiplasmodial activity of EC. The activity of the test doses was estimated using variables like the mean survival time and the % chemosuppression. In comparison to the negative controls, which produced mice parasitaemia levels of 5.300.24%, all tested doses of the leaf extract significantly reduced the mice's levels of parasitaemia. Extract produced parasitaemia reductions of 4.67 ± 0.31, 4.00 ± 0.13, 3.48 ± 0.02, and 2.46 ± 0.15% at dosages of 100, 200, 400, and 800 mg/kg, respectively. The percentage reduction in parasitaemia levels at 100 and 200 mg/kg was equal, with chloroquine showing the largest reduction in parasitaemia at 0.96 ± 0.02. A dose-dependent increase in activity (between 12 and 54%) was observed in the extract's chemosuppressive assay results, which was significantly higher than the results of the negative control (0.00 ± 0.00%) and significantly lower than those of chloroquine (82.00 ± 0.57%), the standard medication used to treat malaria. A dose-dependent chemosuppressive effect has also been noted by other researchers (Adesida et al. 2021; Nureye et al. 2021; Tadege et al. 2022). At 200 mg/kg, the highest percentage of chemosuppression, 53.69 ± 3.97%, was evoked (Table 3). Any extract that produced a chemosuppression level that was above 50% on average can be regarded as active. According to Krettli et al. (2001), this extract had a malaria agent that was only marginally efficacious against the malaria parasite.

Table 3. In vivo Antiplasmodial Efficacy of EC in Suppressive test

Test Doses/Substance (mg/kg)	% Parasitaemia	% Suppression
NC	5.30 ± 0.24^{e}	0.00 ± 0.00^{a}
100	4.67 ± 0.31^{d}	11.98 ± 8.33^{b}
200	4.00 ± 0.13^{d}	24.58 ± 1.05^{b}
400	3.48 ± 0.02^{c}	34.44 ± 2.79^{c}
800	2.46 ± 0.15^{b}	53.69 ± 1.62^{d}
CQ	0.96 ± 0.02^{a}	82.00 ± 0.57^{e}

Keys: The data are presented as mean SEM, n = 5. Tween 80 in normal saline serves as the NC (negative control), whereas chloroquine (10 mg/kg) serves as the PC. Only values with separate superscripts (a, b, c, d, or e) within columns show a significant difference ($p < 0.05$, one-way analysis of variance followed by the Student-Newman-Keul's post hoc test)

While lower than that of chloroquine (26.50 days), the mean survival time induced by the extract at all tested doses rose significantly and dose-dependently compared to the negative control. The survival times of 12.00 and 11.50 days, respectively, at dosages of 100 and 200 mg/kg were reasonably equivalent to those of the negative control (8.33 days). When compared to the usual medication, chloroquine, the survival times of 14.00 and 17.75 days were substantially different ($p < 0.05$) (Fig. 1).

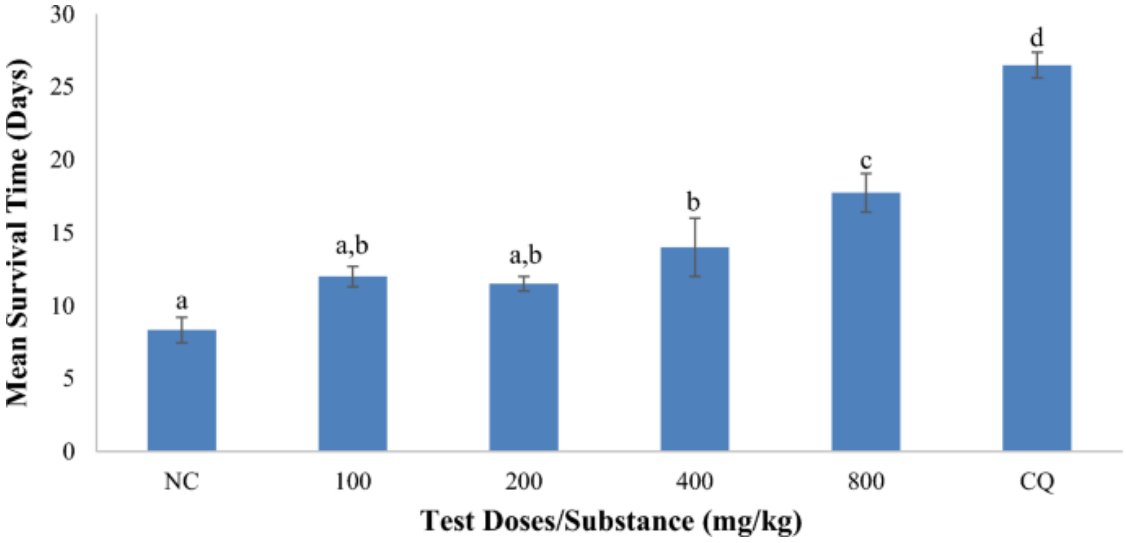

Fig. 1. The effect of EC on mice mean survival time at various doses in chemosuppressive antiplasmodial test is shown in a bar graph. *Keys*: The data are presented as mean SEM, n = 5. Chloroquine (10 mg/kg) and Tween 80 in normal saline make up the NC (negative control). The only values within columns that differ substantially ($p < 0.05$, one-way analysis of variance followed by the Student-Newman-Keul's post hoc test) are those with distinct superscripts (**a**, **b**, **c**, or **d**)

Curative test

Using Rane's test, the therapeutic effect of the methanol extract in mice was identified (Ryley and Peters 1970). The curative activity of the test doses was determined using the metrics (percentage inhibition and mean survival time) frequently utilized in the antimalarial screening of potential drug candidates. The outcomes showed that on Day 3, the activity of the extract at all chloroquine dosages was comparable to that of the negative control (Fig. 2). The activity that the extract at 400 and 800 mg/kg evoked on day four was comparable to chloroquine and dramatically reduced the parasitaemia level when compared to the negative control. The extract significantly decreased all doses' parasitaemia levels on Day 5, compared to the negative control (Fig. 3).

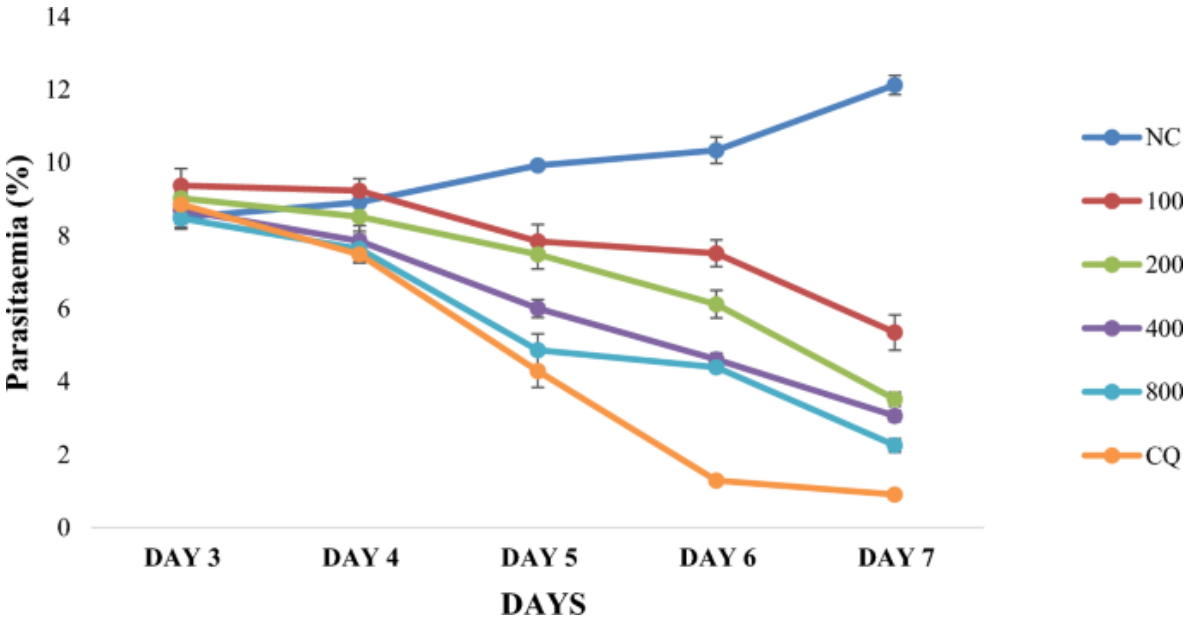

Fig. 2. An in vivo antiplasmodial activity (clearance) graph from Day 3 to Day 7 comparing dosages of EC to the percentage parasitaemia

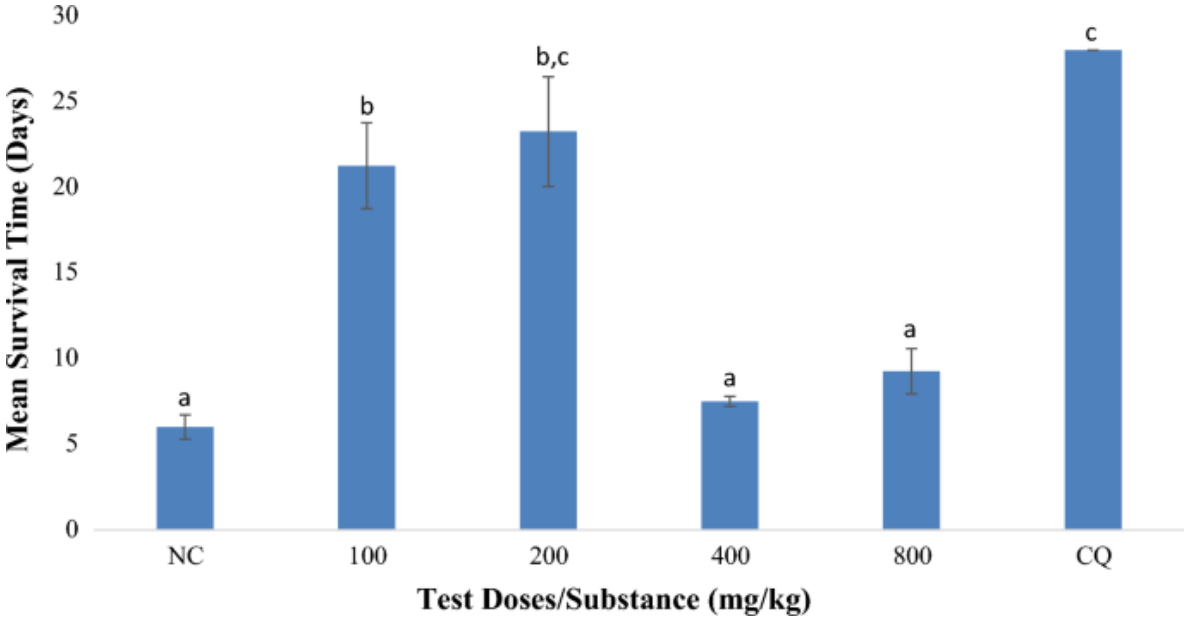

Fig. 3. The mean survival time of EC in mice in a curative antiplasmodial test at various doses is shown in a bar graph. *Keys*: The data are presented as mean SEM, n = 5. Chloroquine (10 mg/kg) and Tween 80 in normal saline make up the NC (negative control). The only values within columns that differ substantially ($p < 0.05$, one-way analysis of variance followed by the Student-Newman-Keul's post hoc test) are those with distinct superscripts (**a**, **b**, **c**, **d** or **e**)

At 100 and 200 mg/kg, it caused a relative reduction in parasitaemia. The level of parasitaemia reduction at 800 mg/kg was comparable to that of chloroquine. Days 6 and 7, when compared to the negative control and chloroquine, the extract dramatically decreased parasitaemia levels across all doses. At 400 and 800 mg/kg on Day 6 and 200 and 400 mg/kg on Day 7, the extract similarly reduced the parasitaemia.

The curative test findings in Table 4 demonstrated that, as compared to the negative control at (p 0. 05 in all cases), EC evoked a dose-dependent increase in percentage clearance. The outcome was consistent with past research on the fruit of *Azadirachta indica* extracted in ethanol (Faloye et al. 2023). The results also showed that the highest average percentage of parasitaemia inhibition was given by chloroquine at a dose of 10 mg/kg (92.510.22%), which was significantly higher than the activity induced by the test extract at the highest dose of 800 mg/kg (81.261.87%). The outcome was comparable to that of a previous report on percentage clearance results of 81.97% obtained by *Commelina latifolia* hydroalcoholic crude extract at 400 mg/kg (Tadege et al. 2022).

Table 4. % chemosuppression in an antiplasmodial test at various doses of EC in Plasmodium berghei-infected mice

Test Doses/Substance (mg/kg)	Days				
	Day 3	Day 4	Day 5	Day 6	Day 7
NC	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
100	-10.25 ± 5.18^{a}	$8.08 \pm 2.19^{a,b}$	20.71 ± 5.45^{b}	27.07 ± 3.76^{b}	55.63 ± 4.67^{b}
200	-6.37 ± 2.76^{a}	$10.11 \pm 3.08^{a,b}$	24.58 ± 3.78^{b}	40.34 ± 4.61^{c}	71.02 ± 1.19^{c}
400	-2.84 ± 6.86^{a}	17.16 ± 3.48^{b}	39.53 ± 2.37^{c}	54.99 ± 3.18^{d}	74.80 ± 0.74^{c}
800	0.50 ± 1.63^{a}	19.18 ± 5.06^{b}	$50.76 \pm 5.19^{c,d}$	57.33 ± 1.27^{d}	81.26 ± 1.87^{d}
CQ	-4.06 ± 2.74^{a}	20.63 ± 3.47^{b}	56.58 ± 5.01^{d}	87.44 ± 0.65^{e}	92.51 ± 0.22^{e}

Keys: The data are presented as mean SEM, n = 5. Tween 80 in normal saline serves as the NC (negative control), whereas chloroquine (10 mg/kg) serves as the PC. The only values within columns that differ substantially ($p < 0.05$, one-way analysis of variance followed by the Student-Newman-Keul's post hoc test) are those with distinct superscripts (a, b, c, d, or e)

Additionally, mice infected with *P. berghei* were cured of their infection by an ethanol extract of *Anarcadium occidentale* leaf at an oral dose of 800 mg (Afolabi and Oluyi 2020). Even at the lowest dose of 100 mg/kg, the extract produced an activity (56%) in the chemosuppressive test that was comparable to the result (54%) for 800 mg/kg. According to the findings (Krettli et al. 2001), extended and higher doses could enhance the efficacy of antimalarial therapeutic effects. The findings suggest that EC has more effective suppressive antiplasmodial agents than restorative ones.

The maximum activity of EC at 200 mg/kg, was comparable to the value found for chloroquine in the antiplasmodial established infection test. It greatly decreased parasitaemia and extended the survival duration of infected mice.

Antiplasmodial activity of the partitioned fractions of EC

Chemosuppressive test

Each proportion of organic solvent showed notable parasitaemia that was distinct from the negative control ($p < 0.05$). While there was no discernible difference between the activities of the various doses of the partitioned fractions at 40 and 80 mg/kg, the aqueous fraction produced a parasitaemia reduction of $2.12 \pm 0.19\%$ compared to the negative control's 8.09 ± 0.60 and was comparable to chloroquine's highest effect (Table 5).

Table 5 % parasitaemia in mice in an antiplasmodial suppressive test of the solvent fractions of EC at different doses

Doses (mg/kg)	% Parasitaemia by solvent fractions ± SEM		
	N-HEX	DCM	AQ
NC	8.09 ± 0.60^{d}	8.09 ± 0.60^{b}	8.09 ± 0.60^{b}
10	4.03 ± 0.18^{c}	2.60 ± 0.30^{a}	2.39 ± 0.05^{a}
20	$3.16 \pm 0.13^{b,c}$	2.45 ± 0.03^{a}	2.25 ± 0.29^{a}
40	$2.34 \pm 0.34^{a,b}$	2.81 ± 0.30^{a}	2.18 ± 0.07^{a}
80	$2.51 \pm 0.19^{a,b}$	2.49 ± 0.10^{a}	2.12 ± 0.19^{a}
CQ	1.71 ± 0.03^{a}	1.71 ± 0.03^{a}	1.71 ± 0.03^{a}

Keys: *N-HEX* n-hexane; *DCM* Dichloromethane; *AQ* Aqueous. The data are presented as mean SEM, n = 5. Tween 80 in normal saline serves as the NC (negative control), whereas chloroquine (10 mg/kg) serves as the PC. The only values within columns that differ substantially ($p < 0.05$, one-way analysis of variance followed by the Student-Newman-Keul's post hoc test) are those with distinct superscripts (a, b, c, d, or e)

The partitioned fractions except n-hexane at 10 mg/kg displayed remarkable % chemosuppression comparable to chloroquine, demonstrating the highest effects (78.92 ± 1.85). The % chemosuppression elicited by the aqueous phase was dose-dependent, while that of n-hexane and dichloromethane was dose-dependent up to 20 mg/kg. The aqueous phase and the various doses of dichloromethane fractions did not differ significantly from one another. The strongest suppressive effect was achieved by n-hexane, dichloromethane, and the aqueous phase at dosages of 40, 20, and 80 mg/kg, respectively (Table 6). The antiplasmodial potency of the solvent fractions of EC can thus be ranked: aqueous > dichloromethane > n-hexane.

Table 6. % chemosuppression in an in vivo antiplasmodial test of the solvent fractions of EC at different doses

Dose	% Chemosuppression by solvent fractions ± SEM		
	NHEX	DCM	AQ
NC	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
10	50.20 ± 3.63^{b}	$67.87 \pm 1.54^{b,c}$	70.49 ± 1.74^{b}
20	60.96 ± 3.64^{c}	$69.76 \pm 2.50^{b,c}$	$72.15 \pm 2.05^{b,c}$
40	$71.09 \pm 2.88^{d,e}$	65.25 ± 4.73^{b}	$73.09 \pm 1.49^{b,c}$
80	69.01 ± 2.04^{d}	$69.27 \pm 2.91^{b,c}$	$73.84 \pm 2.73^{b,c}$
PC	78.92 ± 1.85^{e}	78.92 ± 1.85^{c}	78.92 ± 1.85^{c}

Keys: *N-HEX* n-hexane; *DCM* Dichloromethane; *AQ* Aqueous. The data are presented as mean SEM, n = 5. Tween 80 in normal saline serves as the NC (negative control), whereas chloroquine (10 mg/kg) serves as the PC. The only values within columns that differ substantially ($p < 0.05$, one-way analysis of variance followed by the Student-Newman-Keul's post hoc test) are those with distinct superscripts (a, b, c, d, or e)

The survival time for all the mice treated with the partitioned fractions showed values that were comparable to that of the negative control (15.33 ± 0.88) and significantly lower to that of chloroquine (24.75 ± 3.25) at all tested doses (Fig. 4).

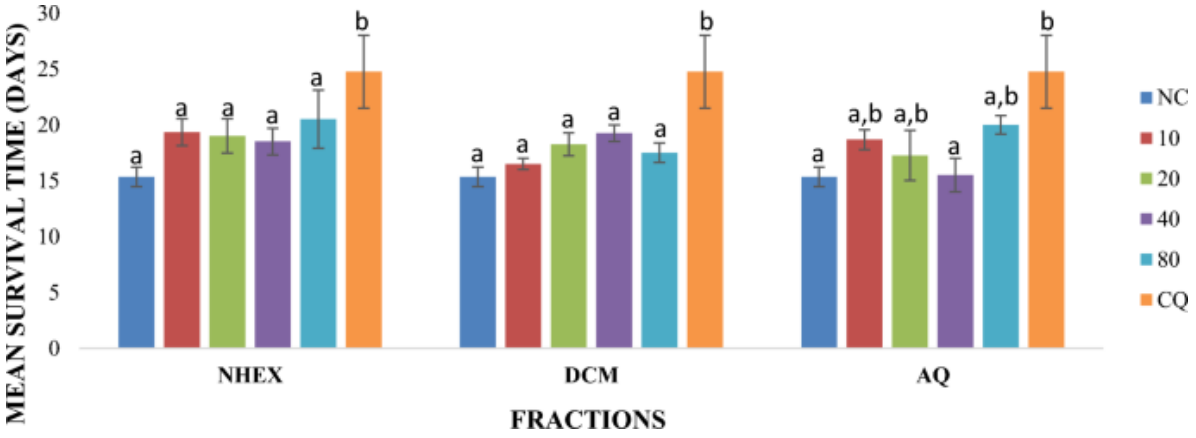

Fig. 4. Average mouse survival times in an in vivo chemosuppressive antiplasmodial test of the solvent fractions of EC are shown in a bar graph. *Keys*: *N-HEX* n-hexane; *DCM* = Dichloromethane; *AQ* Aqueous. The data are presented as mean SEM, n = 5. Tween 80 in normal saline serves as the NC (negative control), whereas chloroquine (10 mg/kg) serves as the PC. The only values within columns that differ substantially ($p < 0.05$, one-way analysis of variance followed by the Student-Newman-Keul's post hoc test) are those with distinct superscripts (**a**, **b**, **c**, **d**, or **e**)

Median effective doses values of EC and its fractions

Using the Microsoft Office Excel 2013 application, the effective median dosage (ED50 and ED90) of EC and its fractions were calculated from a graph of percentage suppression or percentage inhibition versus the doses of the extract (Adesida et al. 2021). The results are shown in Figs. 5 and 6. The effective doses (ED_{50} and ED_{90}) are extrapolated from a graph of % chemosuppression or % clearance in the chemosuppressive or curative antiplasmodial models, respectively. Thus, the doses of the test extract or fractions that will reduce the parasitaemia levels in the treated animals by 50 and 90% are the ED_{50} and ED_{90}, respectively (Adesida et al. 2021; Faloye et al. 2023). According to the results, the ED_{50} and ED_{90} of EC are 548.33 and 1040.01, respectively, in the 4-day suppressive test, while they are 256.18 and 525.31, respectively, in the Rane's Tests (Fig. 5). The ED_{50} and ED_{90} values of chloroquine were reported to be 2.19 and 4.29, respectively, by Adebajo et al. (2013) and Odediran et al. (2014).

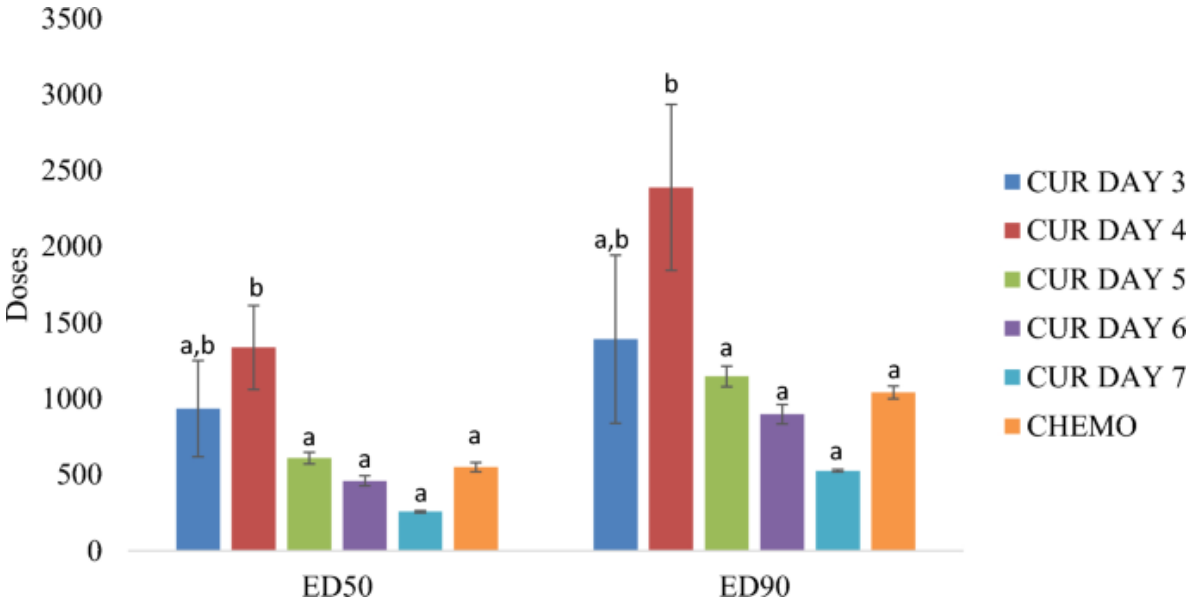

Fig. 5. The 4-Day Suppressive and Rane's Tests median effective doses (ED50 and ED90) of EC are displayed in a bar graph. *Keys*: *EC* methanol extract of *Eucalyptus camaldulensis* leaf, *CUR* Curative model; *CHEMO* Chemosuppressive model. The data are presented as mean SEM, n = 5. The only values within columns that differ substantially ($p < 0.05$, one-way analysis of variance followed by the Student-Newman-Keul's post hoc test) are those with distinct superscripts (**a** or **b**)

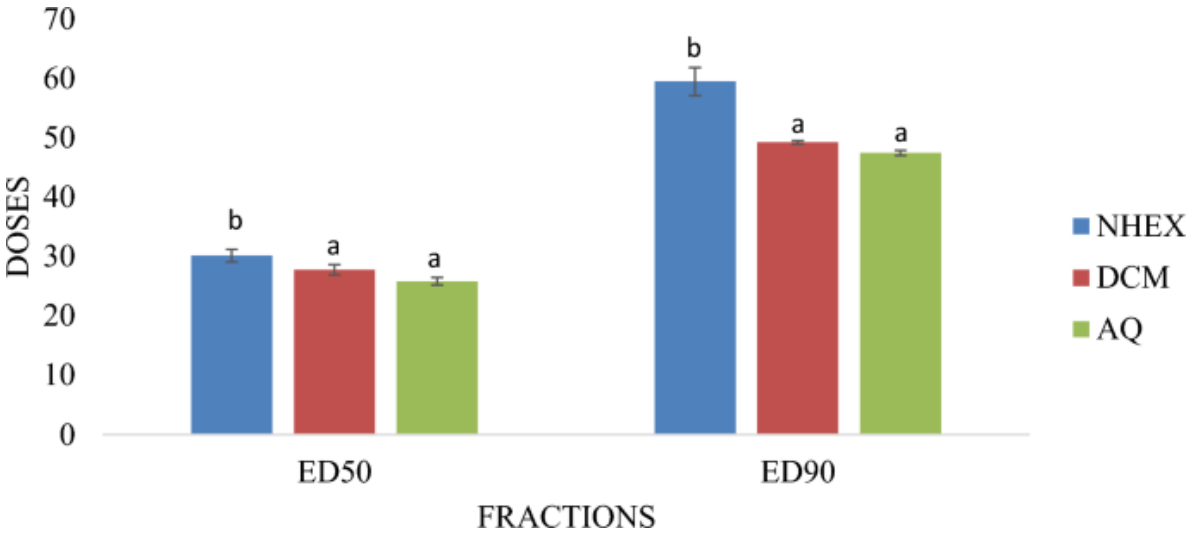

Fig. 6. Median Effective Doses (ED_{50} and ED_{90}) values of the partitioned fractions of EC are displayed in a bar graph. *Keys*: *EC* Methanol extract of *Eucalyptus camaldulensis* leaf, N-HEX: n-hexane fraction; *DCM*: dichloromethane fraction; *AQ*: aqueous phase. The data are presented as mean SEM, n = 5. The only values within columns that differ substantially ($p < 0.05$, one-way analysis of variance followed by the Student-Newman-Keul's post hoc test) are those with distinct superscripts (a or b)

The ED_{50} and ED_{90} of the aqueous fraction gave values of 25.79 ± 0.63 and 47.42 ± 0.44, respectively. Its ED_{50} and ED_{90} was comparable to that of other fractions to that of dichloromethane (27.73 ± 0.85; 49.17 ± 0.28) fractions and significantly ($p < 0.05$), lower than that of n-hexane (30.11 ± 1.07, 59.43 ± 2.39),

The study revealed the antiplasmodial efficacy of EC and its fractions. It showed that increasing the doses of EC extract increased the average percentage of chemosuppressive activity and the mean survival time in the chemosuppressive model. But in the curative model, an increase in dose resulted in an increased and better curative activity but failed to improve the mean survival time. The dose-dependent increase in activity agreed with the earlier similar curative antimalarial work on the plant by Ishaya et al. (2019). The better curative activity could be as a result of prolonged days of drug administration. A high proportion of phenolic compounds, such as quercetin, luteolin, kaempferol, isorhamnetin, phloretin etc., has been reported to be present in the leaves of *Eucalyptus* plants. Quercetin and its derivatives have also been reported to be an excellent antiplasmodial agent (Fentahun et al. 2017). The result of the chemosuppressive test of EC is 24.58% when compared to 200 mg/kg, which is lower than the value of 61.88% reported by Anigboro et al. (2020) for the aqueous leaf extract of the plant using the same model. The result revealed that the aqueous extract of the plant possesses more potent antiplasmodial activity than the methanol extract. This is also supported by the aqueous fraction having the highest activity.

The partitioned fraction gave improved antiplasmodial activity than the crude extract. The improved activity validated the fact that fractionation or purification increases activity. This result was similar to the earlier report on the methanol extract and partitioned fractions of *Plumeria alba* (Adesida et al. 2021). But this is only so for some crude extracts. It has been found that some secondary metabolites work in synergism in some extracts, thereby, partitioning of such will result in reduced activity (Ganesan 2008).

Conclusion

The results showed that EC methanol extract is non-toxic and possesses suitable larvicidal and antiplasmodial activities. The partitioned fractions elicited suitable larvicidal properties, with their activity mainly resident in the n-hexane fraction, while the aqueous fraction showed more significant antiplasmodial potential. This study justifies the ethnomedicinal usage of the plant in malaria therapy. Further haematological activity, isolation and characterization of larvicidal and antiplasmodial compounds are recommended.

Contributions

KOF: conceptualization, supervision, investigation, methodology, validation, data curation, writing–original draft, writing–review & editing. SAA: supervision, investigation, methodology, validation, data curation, writing–original draft, review & editing. SAO: Data acquisition and methodology AHA: Data curation, writing–original draft, writing–review & editing. FGF, OJO and MOO: Supervision, Investigation, Data curation, review and editing. OIB, PAA, SBO, AJO: Methodology, review & editing.

Ethics declarations

Ethical approval

The study protocol was approved by the experimental ethics committee of the Postgraduate College, Obafemi Awolowo University with SCP17/18/H/1272. They were also handled in accordance with the National Institutes of Health (NIH) Guide for the care and use of laboratory animals; (NIH Publication, No. 83-123 (revised), 1985) was followed in the handling caring of the mice.

Conflict of interest

Stephen A. Adesida has no conflict of interest. Samuel A. Oguntimehin has no conflict of interest. Fumilayo G. Famuyiwa has no conflict of interest. Kolade O. Faloye has no conflict of interest. Seun B. Ogundele has no conflict of interest. Oyenike I. Bello has no conflict of interest. Oluyemi J. Oladiran has no conflict of interest. Ayobami J. Olusola has no conflict of interest. Adetola H. Adewole has no conflict of interest. Praise A. Adebayo has no conflict of interest. Maryam O. Oredola has no conflict of interest.

Data availability

All the necessary data supporting the result and conclusion of the study have been incorporated in the manuscript.

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