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GC-MS Analysis and In Vivo Antimalarial Activities of Seed Extract and Solvent Fractions of *Telfairia occidentalis* in *Plasmodium berghei*-infected Mice

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Abstract: *Telfairia occidentalis* Hooke. F. (Cucurbitaceae family), a vegetable whose parts are used for both nutritional and medicinal purposes especially was investigated for anti-malarial activity in mice. The dried seed powder was separately cold extracted in 50% ethanol and gradient solvents (n-hexane, dichloromethane, ethyl acetate and methanol) along polarity gradient to obtained crude ethanol extract and solvents fractions of *T. occidentalis* seed. Based on previously established median lethal dose, the seed extract (138-553 mg/kg) and solvents fractions (276 mg/kg) were investigated for *in vivo* activity against *Plasmodium berghei* infection in mice using suppressive, prophylactic and curative standard models. Gas chromatography-mass spectroscopy (GC-MS) analysis of the active fraction was also done to identify its chemical constituents. The seed extract and fractions (138-553 mg/kg, p.o.) exerted significant ($p<0.05$–0.001) chemosuppressive activity against *P. berghei* infection in suppressive (65.67%; 18.33±3.71 days), prophylactic (55.39%; 17.66±2.18 days) and curative (77.48%; 18.00±1.15 days) tests with methanol fraction having the highest activity. GC-MS analysis of the active methanol fraction revealed the presence of polyunsaturated fatty acids and monoterpenes which have been implicated previously in antimalarial activity of plants. These results revealed that the strong antimalarial potentials of the methanol seed fraction and its phytochemical constituents which can be exploited in the development of antimalarial remedies.

Introduction

Malaria remains one of the major health threats globally. Of the estimated 263 million malaria cases recorded worldwide in 2023, 94% (246 million cases) occurred in Africa. Five African countries; Nigeria (25.9%), the Democratic Republic of the Congo (12.6%), Uganda (4.8%), Ethiopia (3.6%), and Mozambique (3.5%), together accounted for 52% of the global malaria burden (1). Similarly, 95% of the estimated 597,000 global malaria deaths in 2023 were recorded in the WHO African Region. Four African countries accounted for more than 50% of these deaths, with 39.3% of all global malaria deaths in children under five years occurred in Nigeria (1, 2). Although reasons advanced for the upwards trends in malaria cases and deaths included lack of funding and investment, particularly in high-burden African countries, the main reason might not be far from poverty and poor economic condition. Not only are the major artemisinin combination therapies (ACTs) very costly and beyond the reach of the poor populace, but many people are also financially handicapped to access health facilities for treatment. Quite worrisome is the report of artemisinin partial resistance confirmed in Eritrea, Rwanda, Uganda and Tanzania, and suspected in Ethiopia, Sudan, Namibia and Zambia (1). The emerging piperaquine resistance that has emerged in some South American countries (1), which will likely to spread and reduce the efficacy of dihydroartemisinin–piperaquine coupled with the inability of the currently introduced vaccines to provide lasting efficacy and durable protection, poses a serious threat to the control of malaria menace and deadly consequences.

In spite of significant gains achieved in the management of malaria over the past years, the above 2023 malaria data indicate that malaria is a serious threat to lives in Africa, and Nigeria in particular sequel to emergence of partial resistance to artemisinin in some Arican countries (1). Therefore, instigating researchers to search for safe and cost-effective treatments. Medicinal plants provide the reservoirs for potential efficacious antimalarial drugs including the front-line drug, artemisinin and others. Previously we have investigated a number of medicinal plants in Nigeria *in vitro* and *in vivo* (3-8) for the development of potential treatment of malaria.

Telfairia occidentalis Hook, is a fluted pumpkin of the *Cucurbitaceae* family widely consumed as food in Nigeria, especially in the Niger-Delta region and the Eastern part of the country (9). It is a widely consumed vegetable across Nigeria, particularly in the Niger-Delta and Eastern regions, where various meals are prepared from its leaves, stem, and seeds (10). The seeds are very nutritious and are eaten roasted or boiled. The seed has history of being effective in the treatment and prevention of prostrate disorders. The seed extract has been reported to exert antidiabetic (11) cellular antioxidant, immunodulatory, anticancer, antiinflammatory (12), antiplasmodial (13), antioxidant (14), analgesic (14, 15), genotoxic and cytotoxic (16), *in vivo* inhibitory effect alpha amylase and alpha glucosidase (17), antiulcer (18) and antiprostatic (19) activities. Phytochemical studies of the extract have shown the presence of alkaloid, flavonoid, tannins, terpenes, saponin, and cardiac glycosides (20). Some terpenoids and fatty acid esters including 16-octadecenoic acid methyl ester; octadecanoic acid, terpinen-4-ol; trans-β-ocimene and borneol have previously been reported in the seed extract (12). While HPLC characterisation of the seed extract and fractions revealed the presence of eleven flavonoids; kaempferol, catechin, epicatechin, anthocyanidin, naringenin, flavonones, flavones, rutin, naringin, and resveratol in the seed extract and fractions with butanol fraction having the highest concentration. Also, alkaloids such as ribalinidine, ammodendrine, spartein and lunamarin were found in the seed extract and fractions (20). Although earlier studies (13, 20), had reported on antiplasmodial potential of the *T. occidentalis* crude seed extract against *P. berghei* infection in mice, investigation of antimalarial potentials of its solvent fractions to identify the most active fraction has not been carried out. The present study was designed to evaluate the activities of seed extract and fractions of *T. occidentalis* in *Plasmodium berghei*-infected mice and to analyse the active antiplasmodial fraction for possible involvement of volatile phytochemical compounds in the antimalarial activities of the seed extract and fractions using GCMS.

Materials and Methods

Plant Collection and Extraction

Freshseeds of *Telfairia occidentalis* were purchased from Itam market in Itu L. G. A, Akwa Ibom State, Nigeria, in June, 2023. The seeds were previously identified and authenticated by a taxonomist in the Department of Botany and Ecological Study, University of Uyo, Uyo, Nigeria. Herbarium specimens (UUPH 1(b)) were deposited at Department of Pharmacognosy and Natural Medicine Herbarium, University of Uyo. The fresh seeds of the plant were dried on laboratory **table for** 2 weeks and reduced to powder. The seeds powder (1 kg) was macerated in 50% ethanol (Sigma-Aldrich, USA) (5000 mL) for 72 h, while the remaining part (2 kg) was successively and gradiently macerated for 72 h in 5000 mL of each of, n-hexane, dichloromethane, ethyl acetate and methanol (Sigma-Aldrich, USA) respectively, along their polarity to give the corresponding gradient fractions for each solvent. The liquid filtrate of crude extract and solvent fractions were concentrated and evaporated to dryness in *vacuo* 40^0C using a rotary evaporator (Yamato scientific co. Ltd, USA). The various yields were calculated and the extract/solvent fractions were stored in a refrigerator at -4^0C, until used for the proposed experiments.

$$\text{Parasitemia}\ (\%) = \frac{\text{Total number of parasitised RBCs}}{\text{Total number of RBCs}} \times 100$$

Equation 1 | where parasitemia (%) is the percentage of infected erythrocytes relative to the total erythrocytes counted.

Microorganism

Chloroquine-sensitive strain of *Plasmodium berghei (*ANKA strain) was obtained from the National Institute of Medical Research (NIMER), Yaba Lagos, Nigeria and maintained by subpassage of blood from infected to healthy mouse once every 7-8 days.

Parasite Inoculation

Each mouse used in the experiment was inoculated intraperitoneally with 0.2 mL of infected blood containing about 1 x 10^7*P. berghei* parasitized erythrocytes collected from an infected-mice with 20-30% parasitemia. The inoculum consisted of 5 x 10^7 *P. berghei* infected erythrocytes per milliliter prepared by determining both the percentage parasitemia and the erythrocytes count of the donor mouse and diluting the blood with isotonic saline in proportions indicated by both determinations (7, 21). Parasitemia was monitored by standard methods; thin blood smears were made on glass slides, fixed using methanol, and stained using Giemsa stain (Sigma, USA), and parasitemia was counted using a microscope (Nikon, UK) and was calculated as a percentage of infected red blood cells (RBCs) relative to the total number of cells in a microscopic field at ×100 magnification according to the formula of Peters and Robinson, 1992 (22) as shown in **Equation 1**.

Experimental Animals

Swiss albino mice (18-25 g), male and female, used in the study were obtained from the University of Uyo's animal house. They were kept in standard plastic cages in a well-ventilated room and left to acclimatized for a period of 10 days before the experiments. The mice were fed on standard pelleted diet and water *ad libitum,* kept under ambient temperature (28±2°C) and illuminated environment of 12:12 h dark/light cycle. The care and use of animals were conducted in accordance with the National Institute of Health Guide for the Care and Use of laboratory Animals (23). Prior ethical approval for the study was obtained from the University of Uyo's Animal Ethics Committee (UU/CHS/IHREC/23/VOL.1/36).

Drug Administration

In this study, extract and fractions dissolved in 12% Tween 80, chloroquine and pyrimethamine (Sigma, USA) dissolved in distilled water were administered orally with the aid of a stainless metallic feeding cannula.

Evaluation of the *In Vivo* Antimalarial Activities of Crude Extracts and Solvent Fractions of *Telfairia occidentalis* Seed:

Evaluation of Suppressive Activities of the Crude Extract and Solvent Fractions of *T. occidentalis* Seed (4-Day Test)

This test was used to evaluate the schizontocidal activity of the crude extract and solvent fractions as well as chloroquine against early *P. berghei* infection in mice. This was done as described previously (24, 25). Forty-five mice were randomly

divided into nine groups of five (5) mice each. On the first day (D_0), the 45 mice were infected with the parasite and randomly divided into various groups based on their body weights. They were administered the crude extract, gradient fractions, chloroquine and distilled water. Based on the previously established LD_{50} of 3460 mg/kg of the seed extract (25), the administered doses were chosen as fractions (1/6.25, 1/12.5 and 1/25) of the LD_{50} and the the mice in groups 1-3 were given 138 mg/kg, 276 mg/kg and 553 mg/kg of crude extract respectively, while groups 4, 5, 6, and 7 were administered 276 mg/kg of *n*-hexane, dichloromethane, ethyl acetate, and methanol solvent fractions respectively. Group 8 was given 5 mg/kg of chloroquine (positive control) and group 9 was given 10 mL/kg of distilled water (negative control) for four consecutive days (D_0-D_3) between 8am to 9am. On the fifth day (D_4), thin films were made from the tail blood. The films were stained with Giemsa stain to reveal parasitized erythrocytes counted out of 500 in a random field of the microscope. The average suppression of parasitemia was calculated (24) as follows: (average % parasitemia positive control – average % parasitemia negative control) / (average % parasitemia negative control). The mean survival time of the mice in each treatment group was determined over a period of 29 days (D_0-D_{28}).

Evaluation of Prophylactic Activities of the Crude Extract and Solvent Fractions of *Telfairia occidentalis* Seed

This was evaluated using a standard method described by Peters (1965) and Okokon *et al.* (2019) (26, 7). The mice were randomly divided into nine groups of five mice per group. Groups 1-3 were given 138, 276 and 553 mg/kg of crude extract respectively, groups 4, 5, 6, and 7 were given 276 mg/kg of *n*-hexane, ethyl acetate, dichloromethane, and methanol solvent fractions respectively, group 8 was given 1.2 mg/kg of pyrimethamine (positive control) and group 9 was given 10 mL/kg of distilled water (negative control). Administration of the extract and fractions continued for three consecutive days (D_0-D_2). On the fourth day (D_3), the mice were inoculated with *P. berghei.* The parasitemia level was assessed by blood smears 72 h later. The mean survival time of the animals was calculated over a period of 29 days.

Evaluation of the Curative Activities of the Crude Extract and Solvent Fractions of *Telfairia occidentalis* Seed

This test was according to standard method (27) and used to evaluate the schizontocidal activity of the extract, fractions and chloroquine in established plasmodial infection. *P. berghei* was injected intraperitoneally into forty five (45) mice on the first day (D_0). Seventy two h later (D_3), the mice were divided into nine groups of five mice per group. Groups 1-3 were given different doses of extract, 138, 276 and 553 mg/kg respectively, groups 4-7 were given 276 mg/kg of *n*-hexane, ethyl acetate, dichloromethane, and methanol solvent fractions respectively, group 8 was given 5 mg/kg chloroquine (positive control) and group 9 was given 10 mL/kg distilled water (negative control). The crude extract, gradient extracts and chloroquine were administered once daily for 5 days. Giemsa stained thin smears were prepared from tail blood samples collected on each day of treatment to monitor the parasitemia level. Rectal temperatures of the mice were taken on days 0, 3, 5, and 7. The mean survival time (MST) of the mice in each group was determined over a period of 29 days (D_0-D_{28}).

Gass Chromatography Mass-Spectrometry Analysis

Gas chromatography-mass spectrometry (GC-MS) data of the active solvent fraction (methanol) were recorded on an An Agilent 6890N gas chromatography equipped with an autosampler connected to an Agilent Mass Spectrophotometric Detector. One microlitre of the sample was injected in the pulsed spitless mode onto a 30 m x 0.25 mm ID DB 5MS coated fused silica column with a film thickness of 0.15 micrometre. Helium gas was used as a carrier gas, and the column head pressure was maintained at 20psi to give a constant of 1ml/min. Other operating conditions were preset. The column temperature was initially held at 55 ^{0}C for 0.4min, increased to 200 ^{0}C at a rate of 25^{0}C/mins, then to 280^{0}C at a rate of 8^{0}C/mins and to a final temperature of 300^{0}C at a rate of 25^{0}C/mins, held for 2mins. The identification time was based on retention time. Components with lower retention time elute first before the ones of higher retention time. The fractions were directly injected in *n*-hexane as described previously (28). The phytochemicals were identified by comparison of spectra in the NIST 2011 database.

Statistical Analysis

Data collected were analyzed using one way analysis of variance (ANOVA) followed by Tukey's multiple comparison post-test (Graph pad prism software Inc. La Jolla, CA, USA). Values were expressed as mean ± SEM and significance relative to control were considered at $p^{\wedge}0.05$.

Result and Discussion

Yields of Crude Extract and Solvent Fractions

The percentage yields of the crude extract and solvent fractions were; crude-21.03%, n-hexane- 1.6%, DCM -2.82%, ethyl acetate -1.04%, methanol -2.1%.

Suppressive Activities of Ethanol Crude Extract and Solvent Fractions of *Telfairia occidentalis* Seed

The seed extract and its solvent fractions exerted dose-dependent reductions in parasitemia of the treated mice in various groups. The reductions were statistically significant ($p<0.05$) at all doses administered when compared to the negative control. The methanol solvent fraction exerted the highest suppressive effect (65.67 %) with MST of 18.33±3.71 days followed by n-hexane fraction (43.27 %) with MST of 12.00±1.00 days. Although the extracts' treatments resulted in considerable prolongation of the mean survival time (m.s.t) of the infected mice, these were only significant ($p<0.01$-0.001) in the groups treated with the middle and high doses of the crude extract (276 and 553 mg/kg), ethyl acetate and methanol solvent fractions, when compared to the negative control (**Table 1**).

Prophylactic/Repository Activities of Ethanol Crude Extract and Gradient Extractof *Telfairia occidentalis* Seed

The seed crude extract exerted dose-dependent reductions of parasitemia in the treated groups with the highest dose (553 mg/kg) producing the highest chemosuppression of 55.39 % and m.s.t value of 17.66±2.18 days. These reductions were statistically significant relative to the negative control ($p<0.001$). The dichloromethane fraction

caused a chemosuppressive effect of 46.55% with MST value of 14.33±0.33% followed by ethyl acetate fraction with chemosuppressive activity of 39.21 % and m.s.t value of 13.33 ±0.66 days which was not significant (p>0.05) when compared with negative control. The standard drug, pyrimethamine, 1.2 mg/kg exhibited a prophylactic activity of 81.82 % (**Table 2**).

Antiplasmodial Effect of Ethanol Crude Extract and Fractions of *Telfairia occidentalis* on Established Infection

There were progressive dose-dependent reductions of parasitemia in all the extract-treated groups relative to negative control. These reductions were statistically significant relative to the negative control (p<0.001; **Figure 1**). The methanol fraction had the highest activity with chemosuppressive effect of 77.48 % on day 7, this was lower compared with that of the standard, chloroquine, 93.17%. The seed extract demonstrated significant (p<0.05-0.001) prolongation in the mean survival time of the animals. The groups treated with methanol fraction had a longer mean survival time, 18.00 ± 1.15 days followed by those of crude extract (553 mg/kg) treated mice, 15.66 ± 1.45 days. These were less than that of the standard drug, chloroquine (29.83 ± 0.16 days; **Figure 2**).

Effect of Seed Extract/Fractions on Rectal Temperatures of Infected Mice

Administration of the seed extract and fractions of *T. occidentalis* as well as chloroquine to *Plasmodium berghei*-infected mice in curative test did not cause any significant difference (p>0.05) in the rectal temperatures of the treated mice when compared with that of controls (**Table 3**).

GC-MS Analysis

GC–MS analysis of the methanol fraction revealed the presence of pharmacologically active compounds such as hexanoic acid, 5,6-dibromo-6-[p-chlorophenyl]-2,4-dioxo-, ethyl ester; octanoic acid; palmitic acid, 9-hexadecenyl ester (Z)-; oleic acid, 2-(1-octadecenyloxy)ethyl ester (E)-; 9,12-octadecadienoic acid (Z,Z)-, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester; α-carotene, 7,8-dihydro-3,3',19-trihydroxy-8-oxo-, triacetate, all-trans-; and eicosanoic acid, octadecyl ester, among others (**Table 4**; **Figure 3**).

Discussion

The seeds of *T. occidentalis* are used locally as food and to treat various diseases such as malaria, diabetes, fever, inflammatory diseases and pain among others. This work was designed to confirm and authenticate the antimalarial potentials of the seed extract and solvent fractions of *T. occidentalis* in order to provide scientific basis for its usage in traditional medicine. The seed extract and solvent fractions of *T. occidentalis* were investigated for antimalarial activity against rodent malaria parasite, *P. berghei* infection in mice using standard *in vivo* models. The extract/fractions significantly reduced the parasitemia in suppressive, prophylactic and curative models in a dose-dependent fashion with methanol fraction exerting the highest suppression and curative activities, while DCM fraction exerted prominent prophylactic activity confirming the antimalarial potential of the seed extract.

The extract and fractions also prolonged the MST of the mice in different models suggesting that they were able to offer certain degree of protection to the mice. This activity could have resulted from plasmodicidal or plasmodistatic activity of the extracts. This further confirms and validates the use of the seed decoctions as malarial remedy. The slight variation in the activities of the extract and fractions suggests the involvement of immunostimulating activity which may be due to the phytochemical constituents in these extract/solvents. Tannins and fatty acids such as linoleic acids have been reported to possess immune-stimulating properties (29, 30).

The strong suppressive activities of the extract and fractions on the development of *Plasmodium berghei* parasitemia as observed during in the study, suggests that the extract affect the erythrocytic stages of the parasite (31). The inability of the extract and fractions to give complete protection to the infected mice in most cases could have resulted from low doses (138 - 553 mg/kg) used, short half-life/ duration of action of the extracts due to rapid biotransformation processes and subsequent elimination (31). Thus, resulting in further development and multiplication of the parasites as well as short MST of the treated mice as observed in this study. However, these results corroborate previous reports on antimalarial activities of the leaf, seed and root extracts of *T. occidentalis* (32, 13) where significant suppressive, prophylactic and curative activities were recorded. These results validate the use of the seed of *T. occidentalis* decoctions as malarial remedy. In this investigation, *in vitro* study, isolation and characterisation of pure compounds from the active fraction could not be carried out to confirm the activities observed in the *in vivo* study.

Okokon *et al*. (12) reported the presence of polyunsaturated fatty acids such as hexadecanoic acid; 16-octadecenoic acid methyl ester; 9,12-octadecadienoyl chloride (Z,Z); 9-octadecadienoic acid (Z)-, 2,3-dihydroxypropyl ester; octadecanoic acid; hexadecanoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester; linoleic acid ethyl ester; and hexadecanoic acid methyl ester, as well as monoterpenes such as α-phellandrene; α-campholene aldehyde; terpinen-4-ol; trans-β-ocimene; and borneol in the seed extract. Also, in this study, GC–MS analysis of the active antiplasmodial methanol fraction revealed the presence of polyunsaturated fatty acid compounds such as hexanoic acid, 5,6-dibromo-6-[p-chlorophenyl]-2,4-dioxo-, ethyl ester; octanoic acid; palmitic acid, 9-hexadecenyl ester (Z)-; oleic acid, 2-(1-octadecenyloxy)ethyl ester (E)-; 9,12-octadecadienoic acid (Z,Z)-, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester; α-carotene, 7,8-dihydro-3,3',19-trihydroxy-8-oxo-, triacetate, all-trans-; and eicosanoic acid, octadecyl ester, among others (**Figure 4**).

Previous studies of the seed extract and fractions by HPLC indicted flavonoids as the main antiplasmodial agents and revealed the presence of eleven flavonoids; kaempferol, catechin, epicatechin, anthocyanidin, flavonones, flavones, naringenin ,rutin, naringin, and resveratol in the extract and fractions (20). Moreso, alkaloids such as spartein, ribalinidine lunamarin and ammodendrine were found to be present in high concentration in a butanol fraction of the seed extract (20). These compounds may be responsible for the observed activities of the extracts. Some secondary metabolites of plants such as alkaloids, flavonoids and triterpenoids have been reported to have antiplasmodial properties (33-35). Polyunsaturated fatty acids (PUFAs) such as hexadecanoic acid, methyl ester, 9,12-octadecadienoic acid methyl ester

(linoleic acid), 9,12,15-octadecatrienoic acid, methyl ester (linoleic acid), and 9-octadecenoic acid as found in the active antiplasmodial fractions in this study have been implicated in antiplasmodial activity and this activity has been reported to increase with the degree of unsaturation (36-42). Also, flavonoids in the seed extract have been shown to possess significant antiplasmodial activity against chloroquine sensitive and resistant strains of *P. falciparum* (43-45). Antioxidant property of flavonoids has been suggested to be responsible for its antiplasmodial activity (46, 44), as elevated free radical levels are common features of malaria disease and are implicated in severe malaria complications. Scavenging of these free radicals could be one of the mechanisms of action of this extract. Other proposed mechanisms of antiplasmodial activity for flavonoids are chelation of nucleic acid base pairing of the parasite (47), modulation of host immunity to tackle disease and inhibition of plasmodial enoyl-ACP reductase (FAB I enzyme) – a key regulator of type II fatty synthases (FAS-II) in *P. falciparum* (48, 49) and binding to parasite's serinethreonine kinase with high affinity thereby affecting its development (50). Furthermore, terpenes and their derivatives, monoterpenes and sesquiterpenes, have been implicated in antiplasmodial activity of many plants (34, 35). Monoterpenes such as limonene, trans-β-ocimene and α-pinene found in the dichloromethane fraction of the seed extract have been implicated in endoperoxidation leading to plasmocidal activity (51).

The results from the GCMS analysis of the active fraction revealed the presence of fatty acid esters and monoterpenes, compounds which show active antioxidation (52, 53, 29, 54). The above-mentioned compounds in the extract and active fractions, maybe responsible for the observed antiplasmodial activities in this study.

Fever is one of the cardinal symptoms of malaria especially in humans. However, *P. berghei* infection in mice is reported to be associated with hypothermia rather than pyrexia (55). Results of rectal temperatures of the infected mice in this study (curative test), showed that there was no significant difference between the mean temperature values of both the treated and untreated infected mice before and after treatment, suggesting that the mice were hypothermic. This hypothermia in mice may have resulted from the serious physiological effects of the malaria parasite on the host, leading to body heat loss and ultimately death of mice (56). Consequently, carbohydrate, lipid, and protein metabolisms of the host are affected negatively by malaria parasite (57, 58). Decreases in metabolic rates of the *P. berghei*-infected mice have been correlated with decreased body temperatures of mice (59). The extract/fractions however, were unable to attenuate these processes and hence the resultant hypothermia.

Conclusion

The findings of this study further suggest that seed extract and fractions of *T. occidentalis* possess antimalarial potentials which is due to the activities of its phytochemical constituents especially the polyunsaturated fatty acids among others. This confirms and authenticates its use as malarial remedy in folkloric medicine.

Declarations

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Conflict of Interest

The authors declare no conflicting interest.

Data Availability

The data generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Statement

All animal experiments were approved by the Animal Research Ethics Committee of the University of Uyo and conducted in accordance with relevant guidelines and regulations.

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Additional Information

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