Original Research Article

Potential use of *Terminalia superba*Engler & Diels (Combretaceae)against cerebral malaria

ABSTRACT

Cerebral malaria is one of the most severe complications of malaria, primarily caused by *Plasmodium falciparum*. The search for alternative or complementary treatments to classical antimalarial therapies is crucial in light of the emergence of drug resistance. *Terminalia superba*, a tropical medicinal plant, has traditionally been used to treat various diseases, including those with parasitic etiology. This study aims to evaluate the potential of *Terminalia superba* in managing cerebral malaria. Cytotoxicity and neuroprotection tests were conducted on neurons using the MTT method. The activity against neuroinflammation was assessed through the LPS (Lipopolysaccharide) test, measuring cytokines TNF-α, Interleukin 8, Interleukin 6, and Interleukin 1β. The ethanolic extract of *Terminalia superba* exhibited non-toxic activity on neurons with an IC_{50} greater than 200 µg/mL. This extract protected astrocytes against oxidative stress induced by H_2O_2 (500 µM). Cell survival increased from 35% to 45%, 59%, 61%, and 73% at concentrations of 3.125, 12.5, 50, and 200 µg/mL, respectively. *Terminalia superba* also reduced the overproduction of cytokines by LPS-stimulated neurons, demonstrating its efficacy against neuroinflammation.

The ethanolic extract of *Terminalia superba*, in addition to being non-cytotoxic, possesses antioxidant and anti-inflammatory properties on neurons. These properties could play an important role in the management of cerebral malaria.

Keywords: Astrocytes, Cerebral malaria, Cytokines, Plasmodium falciparum, Terminalia superba

1. INTRODUCTION

Malaria is a devastating infectious disease that leads to significant mortality and morbidity [1]. In addition to severe malaria-related anemia, cerebral malaria is one of the most serious lethal complications of infection with *Plasmodium falciparum*. Cerebral malaria accounts for 1 to 10% of infections and affects both adults and children. In endemic areas of sub-Saharan Africa, there are between 1 and 12 cases of cerebral malaria per 1000 children per year,

representing approximately 10% of pediatric hospitalizations [2]. The lethality rate is 18.6%, with about 3000 sub-Saharan children dying each day due to this disease [3]. The main mechanisms involved in the genesis of cerebral malaria include the sequestration of parasitized red blood cells in cerebral capillaries, excessive production of pro-inflammatory cytokines, and thrombosis of microvessels, leading to abnormalities in the endothelial barrier. These cytokines and metabolic products exacerbate the loss of red blood cell deformability, resulting in oxidative stress [4]. Currently, curative treatment relies on artesunate administered intravenously as an emergency measure for all patients (adults, pregnant women, and children) [5]. However, individuals who survive fromcerebral malaria may develop transient or permanent neurological sequelae, leading to cognitive impairments. These cognitive deficits primarily arise from the inability of standard antimalarial treatments to prevent neuronal death in brain regions associated with cognition. Therefore, there is an urgent need for an effective antimalarial drug capable of preventing these cognitive deficits.Here we provide insights on the effect of *Terminalia superba* extracts for the management of experimental cerebral malaria.

2. MATERIAL AND METHODS

2.1 Plant Materials

Hydro-ethanolic extract of *Terminalia superba* was prepared from dried barks collected in malaria-endemic region (Agboville). Sample was authenticated at the National Herbarium (CNF) and stored under standardized conditions.

2.2 Cell Culture

Human neuroblastoma cells (SH-SY5Y-neurons) were used as a model for neuronal toxicity and neuroinflammation.

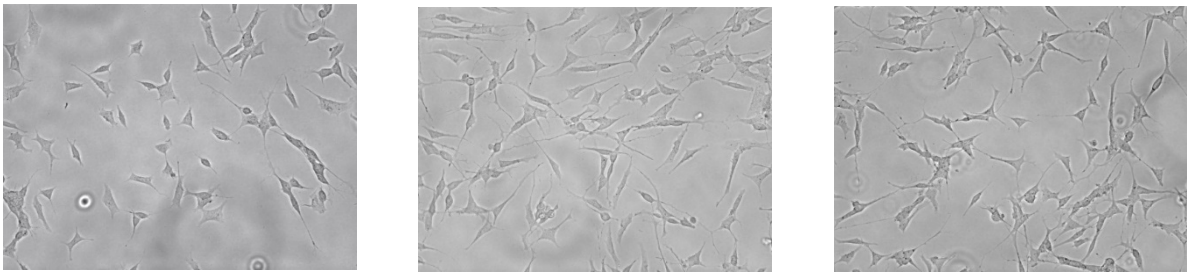
2.3 Preparation of plant extracts

The plant samples were then dried in shade left over for 20 days and powdered with the help of grinder. Powder was extracted according to Zihiri &Kra[6] as follows: One hundred grams of powder were macerated in ethanol 70% during 48 hours. The obtained homogenate was filtered successively on cotton then on Whatman paper 3 mm. The filtrate is first reduced using a rotary evaporator BÜCHI type at 50°C, then collected brown paste is lyophilized. We obtained ethanolic extract.

2.4Neurotoxicity essay

2.4.1 Differentiation of SH-SY5Y cells to SH-SY5Y-neurons

The SH-SY5Y cells provided by the National Brain Research Centre (NBRC) of India were maintained in continuous culture for at least one week (Fig. 1) For differentiation, the old SH-SY5Y cell culture media was removed and replaced with a new MEM media containing 10 µM retinoic acid. Maintenance of the culture by half media change every alternate day (media containing retinoic acid) was done for six days to obtain mature neurons [7].



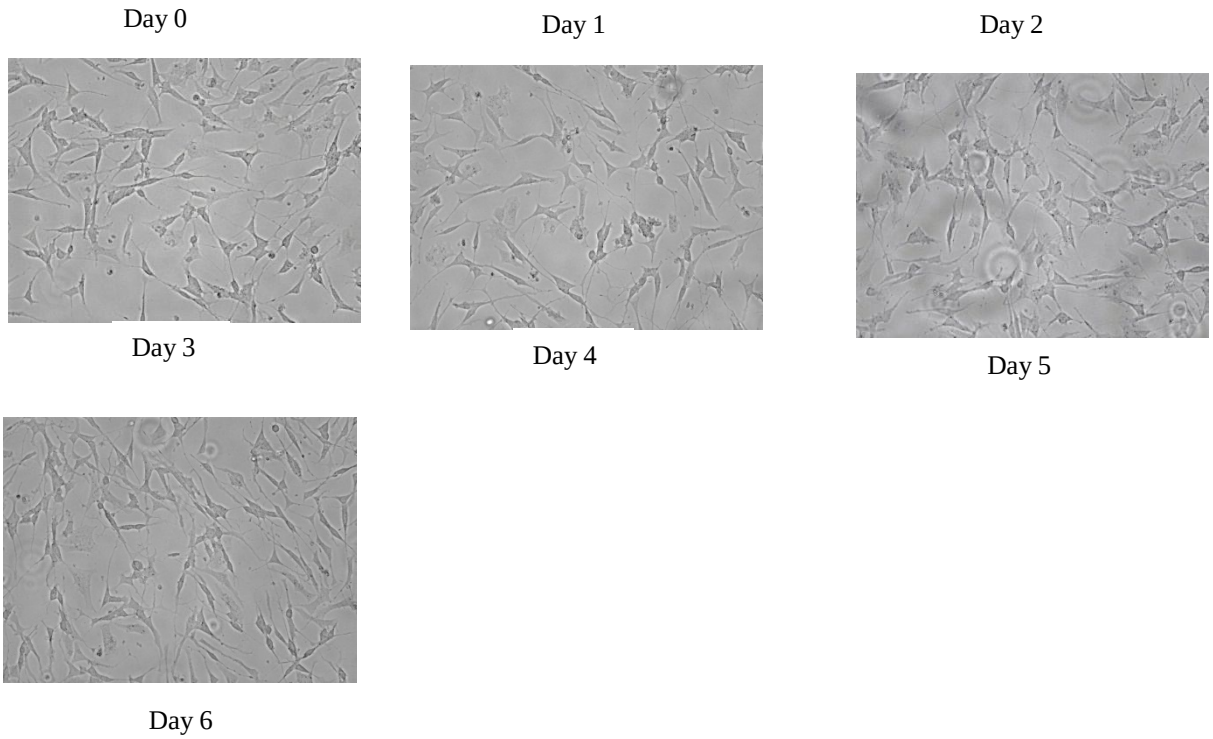


Fig. 1. Differentiation SH-SY5Y to SH-SY5Y-Neurons

2.4.2Treatments

Human SH-SY5Y neurons were cultured in 96-well culture plates at a density of 15,000 cells/well, and incubated at 37°C for 24 h. When Cells were approximately 80% confluent, media was removed and replaced by the different concentrations of *T. superba* : 200, 50, 12.5 et 3.12 µg/mL (100 µL per well). Plate was incubated for 48 h at 37°C.

2.4.3 MTT Assay for assessing Cell Viability

After appropriate time intervals, the media was removed and replaced by 100 µL growth medium with 0.5 mg/mL MTT, and the plates were incubated for an additional 3 h at 37°C. Subsequently, the supernatant was removed and replaced by 100 µL of solubilization solution (50% DMF and 20% SDS) to dissolve the formazan crystals. The optical density (OD) was measured at 570 nm using a 96-well multiscanner autoreader[8]. The results were presented as a percentage of viable cells as compared to the control.

2.5 Protective effect of *Terminalia superba* against H_2O_2 injuries

Cells were seeded into 96-well culture plates at a density of 15,000cells/well. Twenty-four hours after seeding, cells were then pretreated for 1 h with our extract diluted in serum-free media at concentrations of 200, 50, 12.5 et 3.12 µg/mL. The treated cells were then challenged with 500 μM $H_2O_2$for 4h. Then H_2O_2 was removed and replaced by 100 µL

growth medium with 0.5 mg/mL of MTT was added to all wells and allowed to incubate in the dark at 37°C for 3h. The amount of MTT formazan product was determined by measuring absorbance using a microplate reader at 570 nm.

2.6 Anti-neuroinflammatory activity of *Terminalia superba*

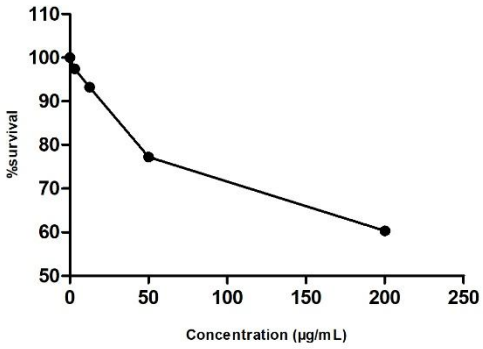
The culture medium was collected, and the levels of IL-1β, IL-6, IL-8 et TNF-α present in each sample were determined using a commercially available kit from BioLegend (San Diego, CA, USA). The assay was performed according to the manufacturer's instructions.

2.7 Statistical analysis

The data was analyzed by one-way ANOVA followed by turkey's test and $P < 0.05$ was used to indicate the statistically significant difference. GraphPad Prism 5 was used to perform the tests.

3. RESULTS AND DISCUSSION

The ethanolic extract of *Terminalia superba* showed an IC_{50} greater than 200 µg/mL (Figure 2). When combined with 500 µM of H_2O_2, the extract demonstrated neuroprotective activity on neurons, with cell survival rates of 45% at 3.125 µg/mL ; 59% at 12.5 µg/mL ; 61% at 50 µg/mL ; and 73% at 200 µg/mL, while H_2O_2 alone resulted in a survival rate of 35% (Fig. 3). The concentration of 50 µg/mL of *Terminalia superba* led to a reduction in the overproduction of cytokines by LPS-stimulated neurons. The cytokine levels decreased from 2291 to 1792 pg/mL for IL-1β, from 5918 to 4439 pg/mL for IL-6, from 11659 to 8760 pg/mL for IL-8, and from 4660 to 2586 pg/mL for TNF-α. The normal cytokine values are 1631, 4804, 6881, and 2593 pg/mL respectively for IL-1β, IL-6, IL-8, and TNF-α (Fig. 4).



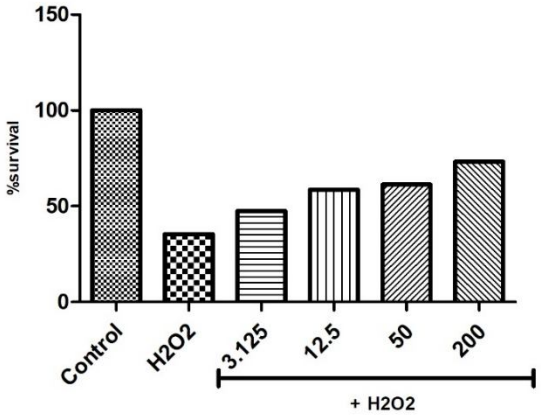


Fig.2. Cytotoxicity of *T. superba* **Fig. 3. Neuroprotection of *T. superba***

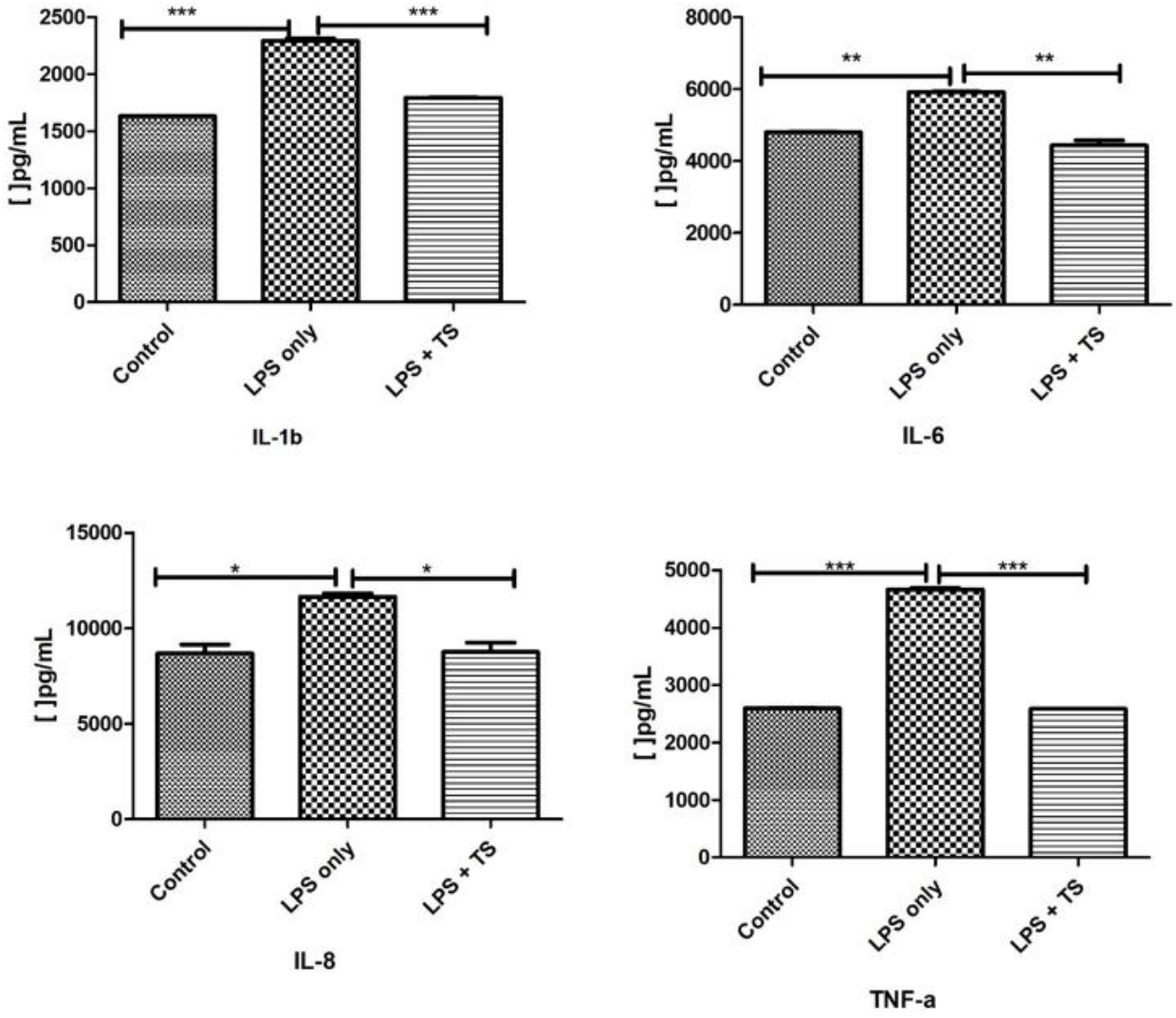


Fig. 4. Anti-neuroinflammatory activity of *Terminalia superba* ((triplicate experiments were executed (P < 0.05))

Cerebral malaria has a high mortality rate, but the understanding of the mechanisms leading to death remains unclear. Factors such as inflammatory cytokines [9, 10], oxidative stress, markers of endothelial activation, coagulation dysfunction [11] and total parasitic load [12]have all been implicated, and combinations of these biomarkers may enhance predictive value [13]. In this research, the objective was to elucidate whether the ethanolic extract of *Terminalia superba* exhibits anti-inflammatory and antioxidant activities useful for resolving cerebral malaria. During malaria, the initial immune responses (oxidative and inflammatory) induced by monocytes are crucial for controlling parasite multiplication. However, excessive and inappropriate activation of the immune system is detrimental to the host and contributes to the severe form that can lead to death [14]. The anti-inflammatory properties of our extract were determined *in vitro* in a non-malarial context. After stimulation with LPS (lipopolysaccharide), treatment with *Terminalia superba* resulted in a significant inhibition of

the overproduction of pro-inflammatory cytokines TNF-α, IL-1β, IL-6, and IL-8. These results align with those of Camara *et al.*[15], who reported that *Terminalia albida* significantly inhibited the expression of TNF-α, IL-1β, IL-6, and IL-12 after LPS/IFNγ stimulation. *Terminalia superba* also demonstrated very interesting *in vitro* antioxidant properties. Furthermore, it neutralized intracellular radical species in SH-SY5Y neurons in a dose-dependent manner. It is widely accepted that oxidative stress is involved in the pathogenesis of severe malaria [16].

3. CONCLUSION

The findings suggest that *Terminalia superba* possesses significant anti-inflammatory and antioxidant activities that could be beneficial in managing cerebral malaria. Further studies are warranted to explore its potential as a therapeutic agent in this severe form of malaria.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Author KGR designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed the literature searches. Author SKD performed the final protocol, followed experiment analysis and read the first draft of the manuscript. Author BA contributed to biological materials. Author DAJ managed the analysis of the study and read the final draft.

CONSENT

It is not applicable.

COMPETING INTERESTS

No competing interests exist.

REFERENCES

1. OMS. Rapport 2023 sur le paludisme dans le monde, Dossier d'information, Données et tendances regionals. 2023; 15 p.
2. The Childhood Acute Illness and Nutrition (CHAIN) Network. Childhood mortality during and after acute illness in Africa and south Asia : a prospective cohort study. Lancet Glob Health. 2022;10(5):e673–e684. doi: 10.1016/S2214-109X(22)00118-8
3. Nguimfack L. Évaluation psychologique d'un cas de neuropaludisme chez l'enfant. *L'Information psychiatrique*. 2019;95(10):837-42.
4. Neveu N. Une complication majeure du paludisme: le neuropaludisme. Sciences pharmaceutiques. 2017; 105p.
5. Dondorp AM, Fanello CI, Hendriksen IC et al. Artesunate versus quinine for treatment of severe falciparum malaria in African children (AQUAMAT): an open-label, randomised trial. Lancet, 2010;376(9753):1647-57.
6. Zirihi GN, Kra AKM. Evaluation del'activité antifongique de *Microglossa pyrifolia* (Larmarck) 0. Kuntze (Asteraceae) « PYMI » sur lacroissance *in vitro*de Candida albicans. Revue de Medécine et de Pharmacopée Africaine. 2003;17:1-18.
7. Azmi NH, Ismail N, Imam MU, Ismail M. Ethyl acetate extract of germinated brown riceattenuateshydrogen peroxide induced oxidative stressinhumanSH-SY5Y

neuroblastoma cells: role ofanti-apoptotic, pro-survival and antioxidant genes. BMC Complementary and Alternative Medicine. 2013;13:177.

8. Simoes LC, Simoes M, Vieira MJ. Biofilm interactionsbetween distinct bacterialgenera isolated from drinking water. Applied and Environmental *Microbiology*.2007;73:6192–6200

9. Grau GE, Taylor TE, Molyneux ME, Wirima JJ, Vassalli P, Hommel M. Tumor necrosis factor and disease severity in children with falciparum malaria.The New England Journal of Medicine.1986; 320:1586-1591.

10. Lyke KE, Burges R, Cissoko Y, Sangaré L, Dao M, Diarra I. Serum levels of the proinflammatory IL-8, IL-10, tumor necrosis factor alpha, and IL-12 (p70) in Malian children with severe *Plasmodium faciparum* malaria and matched uncomplicated malaria or healthy controls », Infection and Immunity.2004;72:5630-5637.

11. Moxon CA, Wassmer SC,Milner DA et al. Loss of endothelial protein C receptors links coagulation and inflammation to parasite sequestration in cerebralmalaria in African children. Blood, 2013;122, 842–851.

12. Ponsford MJ, Medana IM, Prapansilp P, Hien TT, Lee SJ, Dondorp AM. Sequestration and microvascular congestion are associated with coma in human cerebral malaria.The Journal of Infectious Diseases.2012; 205:663-671.

13. Ehrhardt S, Wichmann D, Hemmer CJ, Burchard GD, Brattig NW. Circulating concentrations of cardiac proteins in complicated and uncomplicated *Plasmodium falciparum* malaria. Tropical Medicine & International Health. 2004;9:1099-1103.

14. Dunst J, Kamena F, Matuschewski K. Cytokines and chemokines in cerebral malaria pathogenesis.Frontiers in Cellular and Infection Microbiology. 2017; 7:324.

15. Camara A, Haddad M, Reybier K, Traoré M et al.*Terminalia albida* treatment improves survival in experimental cerebral malaria through reactive oxygen species scavenging and anti-inflammatory properties. Malaria Journal. 2019,18 : 431.

16. Percario S, Moreira DR, Gomes BAQ, Ferreira MES, Gonçalves ACM, LaurindoPSOC. Oxidative stress in malaria.International Journal of Molecular Sciences.2012;13:16346-72.