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Neuroprotective and Anti-inflammatory Effects of *Terminalia superba* Engler & Diels (Combretaceae): A Potential Therapeutic Approach for Cerebral Malaria

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Authors' contributions

This work was carried out in collaboration among 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 provide biological materials. Author DAJ managed the analysis of the study and read the final draft. All authors read and approved the final manuscript.

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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. Ethanolic extracts of *T. superba* were obtained by maceration of the bark of the plant in 70% alcohol. Cytotoxicity and neuroprotection tests were conducted on neurons (SH-SY5Y-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. Further studies should lead to the development of a drug against cerebral malaria based on the bark of *T. superba*.

Keywords: Astrocytes; cerebral malaria; cytokines; plasmodium falciparum; terminalia superba.

1. INTRODUCTION

"Malaria is a devastating infectious disease that leads to significant mortality and morbidity" (OMS, 2023). "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" (The Childhood Acute Illness and Nutrition (CHAIN) Network, 2022). "The lethality rate is 18.6%, with about 3000 sub-Saharan children dying each day due to this disease" (Nguimfack, 2019). "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" (Obeagu, 2024). "Currently, curative treatment relies on artesunate administered intravenously as an emergency measure for all patients (adults, pregnant women, and children)" (Bonsergent et al., 2023). However, individuals who survive from cerebral malaria may develop transient or permanent neurological sequelae, leading to cognitive impairments (Kipre et al., 2018). 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. MATERIALS AND METHODS

2.1 Plant Materials

The bark of Terminalia superba was used in this study. *Terminalia superba* is traditionally used to treat skin infections, malaria, diabetes, high blood pressure, hemorrhoids and gastrointestinal disorders. 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 (2003) 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.

2.4 Neurotoxicity 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 (Camdzic et al., 2023).

2.4.2 Treatments

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.

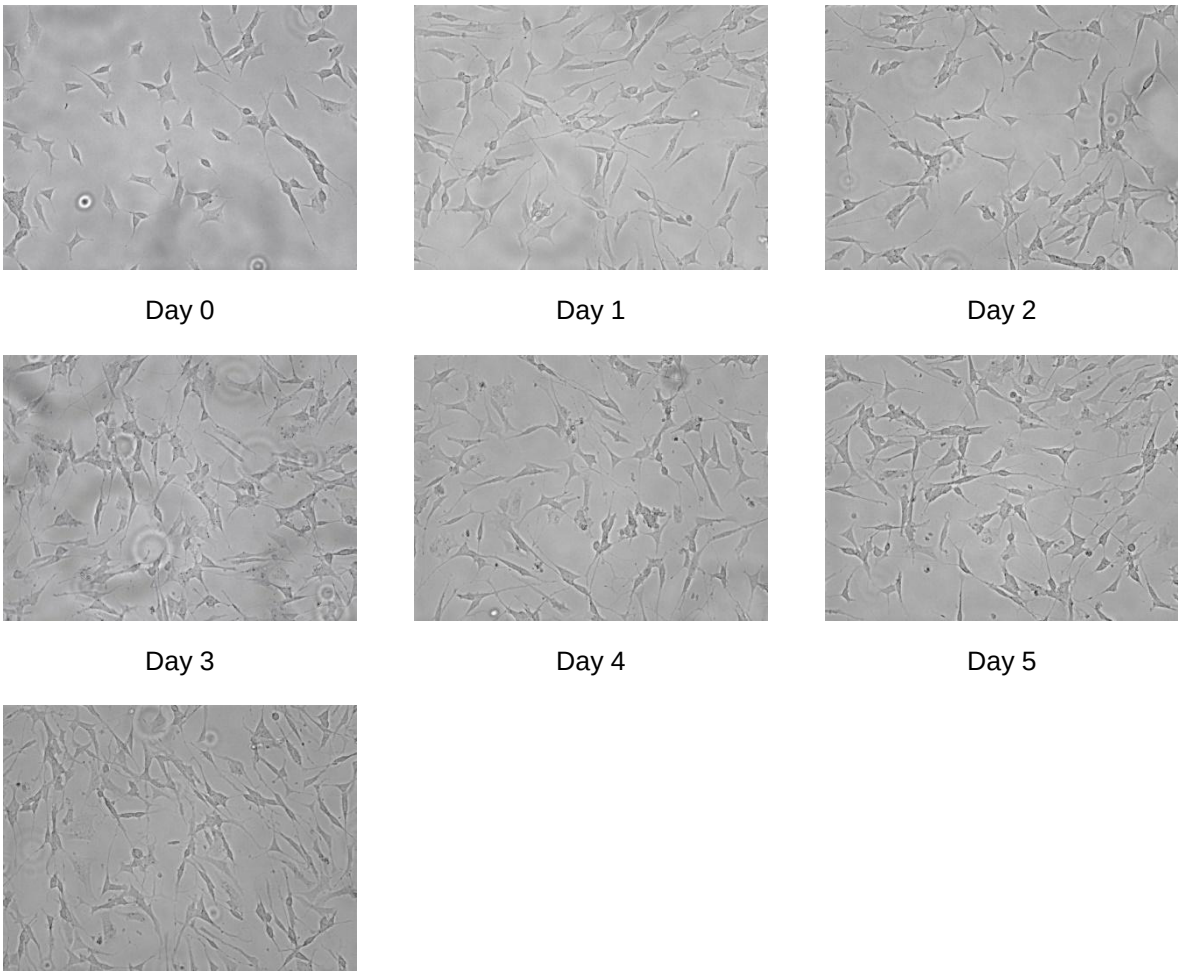

Fig. 1. Stage of differentiation of SH-SY5Y to SH-SY5Y-Neurons

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" (Ghasemi et al., 2021). 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 15,000 cells/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

GraphPad Prism 5 software was used to enter data. Data were analyzed by one-way ANOVA followed by Turkey's test and $P < 0.05$ was used to indicate statistically significant difference.

3. RESULTS AND DISCUSSION

The ethanolic extract of *Terminalia superba* showed an IC_{50} greater than 200 µg/mL (Fig. 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).

Cerebral malaria has a high mortality rate, but the understanding of the mechanisms leading to death remains unclear. Factors such as inflammatory cytokines (Ramachandran and Sharma, 2022; Bensalel and Gallego-Delgado, 2024), oxidative stress, markers of endothelial activation, coagulation dysfunction [(Song et al., 2022) and total parasitic load (Muppidi et al., 2023) have all been implicated, and combinations of these biomarkers may enhance predictive value (Sahu and Mohanty, 2023). "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" (Sahu and Mohanty, 2023). 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.* (2019), 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 (Vasquez et al., 2021).

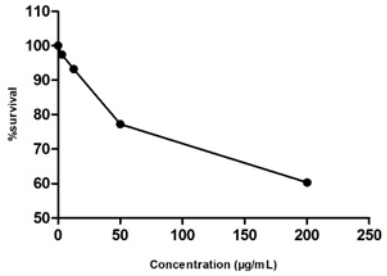

Fig. 2. Cytotoxicity of *T. superba*

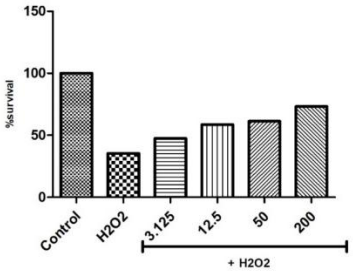

Fig. 3. Neuroprotection of *T. superba* against H_2O_2 injuries

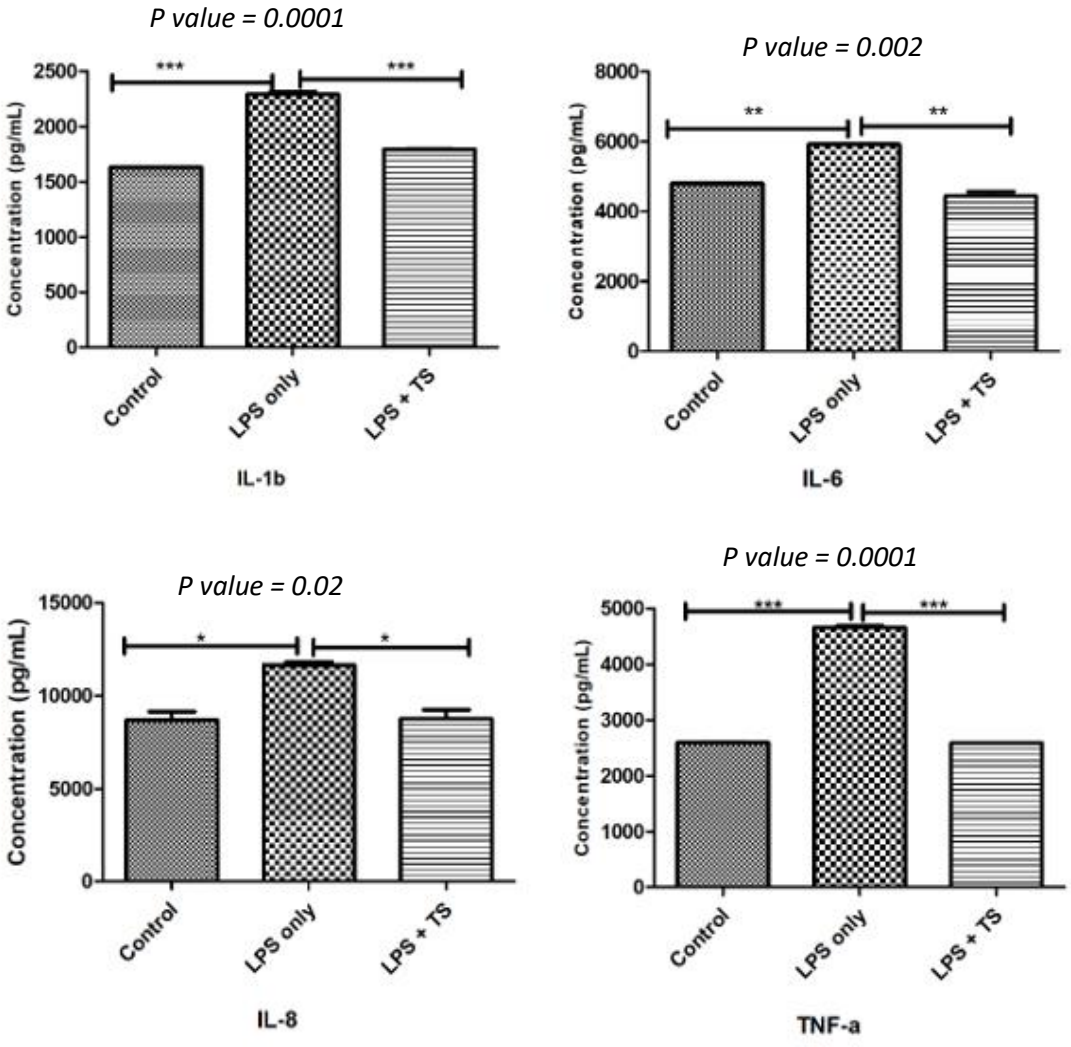

Fig. 4. Anti-neuroinflammatory activity of *Terminalia superba* ((triplicate experiments were executed (P value < 0.05))

The study on Terminalia superba demonstrates its potential to inhibit pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-8) and reduce oxidative stress, mechanisms relevant to severe malaria. However, the translating these results into clinical applications faces challenges, including the complexity of severe malaria pathogenesis, the need for standardized extracts, potential drug

interactions, and the high resource demands of regulatory approval. Future research should focus on animal studies, clinical trials, and exploring combination therapies to advance Terminalia superba from experimental findings to practical therapeutic use.

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.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

CONSENT

It's not applicable.

ETHICAL APPROVAL

It's not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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