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Inhibition of hemozoin-induced neuroinflammation by medicinal plants used for the treatment of malaria in Southwest Nigeria: implications for cerebral malaria

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Shortened title: Inhibition of neuroinflammation by Nigerian plants

Abstract

This study aimed to investigate some antimalarial plants for effects on neuroinflammation. Freeze-dried infusions of the plants were investigated for effects on hemozoin-induced neuroinflammation in BV-2 microglia and levels of inflammatory mediators measured. *Alstonia boonei* (stem bark), *Anacardium occidentale* (stem bark), *Azadiractha indica* (leaves), *Enantia chlorantha* (stem bark), *Khaya senegalensis* (stem bark), *Mangifera indica* (stem bark), and *Nauclea latifolia* (stem bark) produced significant ($p < 0.05$) reduction in $\text{TNF}\alpha$, IL-6, IL-1 β , MCP-1, RANTES and iNOS/NO production in BV-2 microglia stimulated with a synthetic hemozoin (400 $\mu\text{g}/\text{mL}$). Further experiments showed that pre-treatment with 50 $\mu\text{g}/\text{mL}$ of *A. boonei*, *A. indica*, *A. occidentale*, *E. chlorantha* and *M. indica* prior to hemozoin stimulation resulted in inhibition of NF- κ B activation by $>40\%$, while *E. chlorantha*, *K. senegalensis* and *N. latifolia* produced weak activities. Pre-treatment with *A. indica* (50 $\mu\text{g}/\text{mL}$) produced the highest inhibition (58.6%) of hemozoin-induced increased NLRP3 protein expression, while *A. occidentale* (50 $\mu\text{g}/\text{mL}$), *M. indica* (50 $\mu\text{g}/\text{mL}$) and *A. boonei* (50 $\mu\text{g}/\text{mL}$) reduced expression by 54.1%, 49.2% and 47.1%, respectively. Hemozoin-induced increased caspase-1 activity was reduced by *A. boonei*, *A. occidentale*, *A. indica*, *E. chlorantha*, and *M. indica*. These results suggest that *A. boonei*, *A. occidentale*, *A. indica* and *M. indica* produced strong inhibition of hemozoin-induced neuroinflammation through mechanisms involving NF- κ B and NLRP3 inflammasome activation, while moderate activities were produced by *E. chlorantha*, *K. senegalensis* and *N. latifolia*. The outcome of the study provides pharmacological evidence for the potential benefits of the plants as herbal treatments of cerebral malaria symptoms.

Keywords

Traditional African Medicine; Cerebral malaria; Neuroinflammation; Microglia

Introduction

According to the World Health Organisation (WHO), malaria was responsible for approximately 409,000 deaths in 2019, with Africa accounting for more than 94% of global fatalities (WHO 2020). The most severe neurological complication of acute *Plasmodium falciparum* malaria is cerebral malaria (CM), a condition that is responsible for the majority of deaths in African children. In spite of effective antimalarial treatments and intensive care, cerebral malaria causes 15–20% mortality, and survivors may develop long-term neurological deficits (Carter et al. 2005; Boivin et al. 2007; John et al. 2008). Consequently, adjunctive treatments are needed to reduce the mortality of this condition.

Several investigations have now established that in malaria, including CM, there is formation of a metabolic product hemozoin as a result of infection of erythrocytes by *Plasmodium falciparum*. Hemozoin is known to induce inflammation and morphological changes in microvascular endothelium (Medana and Turner 2006; Prato et al. 2011), leading to an increase in blood brain barrier (BBB) permeability, neuroinflammation and neurological sequelae in survivors (Basilico et al. 2003; Tripathi et al. 2009). Similar to these reports, our investigations have revealed that a synthetic form of hemozoin is in fact able to induce neuroinflammation in BV-2 microglia (Velagapudi et al. 2019). Consequently, reducing the CNS effects of hemozoin in CM is a potential adjunctive strategy in reducing the neurological outcomes of the disease.

Plants have a long history of use Traditional African Medicine (TAM) for treating malaria and its complications such as CM. Some of these plants are potential interventions in treating CM due to their ability to reduce processes involved in the

activation of neuroinflammation in the brain. For instance, *Terminalia albida* has been shown to improve survival in experimental CM through its anti-inflammatory action (Camara et al. 2019). Similarly, an extract of *Azadiractha indica* was shown to reduce neuroinflammation and severity of brain oedema in an experimental model of CM (Bedri et al. 2013). Recently, we reported that an extract of *Zanthoxylum zanthoxyloides* attenuated neuroinflammation induced with synthetic hemozoin in BV-2 microglia (Ogunrinade et al. 2021). These reports are encouraging developments, and suggest that further pharmacological evaluation of plants used in TAM for treating malaria and/or CM is warranted.

In this study, we conducted a survey of plants used in the treatment of malaria and/or CM in southwest Nigeria and evaluated freeze-dried infusions of plants with the highest fidelity levels for effects on the increased production of pro-inflammatory mediators in BV-2 microglia stimulated with a synthetic form of hemozoin (HZ).

Material and methods

Materials

Synthetic hemozoin was purchased from InvivoGen (France) and prepared fresh in sterile distilled water.

Collection of plant samples

Field surveys were conducted in Agbegi village, Ikire (Southwest Nigeria), Ile-Igbon (Southwest Nigeria), Beere/Oje area (Ibadan, Southwest Nigeria), Ilesa (Southwest Nigeria) and Ile-Ife (Southwest Nigeria). Traditional medicine practitioners in these communities were approached and informed of the purpose of the study, including research objectives, methods of data collection, and intention to publish data.

Thereafter, semi-structured interviews were conducted to gather information on the

use, preparation, application and properties of herbs used for treating symptoms of malaria and/or cerebral malaria were obtained from each traditional medicine practitioner. Healers were asked specifically for herbal preparations used in treating the usual symptoms of malaria (fever, aches, pains), in addition to cerebral malaria specific symptoms such as coma and seizures.

The Fidelity Level (FL) which is the ratio between the number of informants who independently suggested the use of a plant for malaria/cerebral malaria and the total number of informants who mentioned the plant for any use was calculated using the

$$\text{formula: } FL = \frac{Np}{N} \times 100$$

Np : is the number of informants that report a use of a plant species to treat malaria and/or cerebral malaria

N : the number of informants that used the plants as a medicine to treat any given disease (Friedman et al. 1986).

Plants with $FL > 50\%$ for the treatment of malaria/cerebral malaria symptoms in these surveys were collected and samples authenticated by a botanist in the Herbarium of Forestry Research Institute of Nigeria, Ibadan. Voucher specimens were prepared and numbers assigned.

Preparation of herbal infusions

Plant samples were air-dried and reduced to powdery forms. Thereafter, infusions were prepared by adding 1 g of dried, powdered samples to 200 mL of boiling distilled water and then left to stand at room temperature for 5 min. Subsequently, infusions were filtered and freeze-dried in a Christ Alpha 2-4 LDplus freeze dryer. Known concentrations of freeze-dried plant materials were prepared fresh in sterile distilled water for pharmacological experiments.

Cell culture

BV-2 mouse microglia cell line (ICLCATL03001) was purchased from Interlab Cell Line Collection (Banca Biologica e Cell Factory, Italy) and cultured in RPMI medium supplemented with 10% foetal bovine serum.

Cell viability

Viability of BV-2 cells stimulated with hemozoin in the presence or absence of plant extracts was measured by the MTT assay. Cells were seeded out in a 96-well plate at 5×10^5 cells/ml and then treated with 25 and 50 $\mu\text{g/ml}$ of freeze-dried plant infusions for 30 min, followed by stimulation with HZ (400 $\mu\text{g/ml}$) for 24 h. Then cell culture medium was removed and replaced with MTT solution (5 mg/ml), followed by incubation for 4 h. Thereafter, 150 μL of MTT solution was removed from each well and replaced with 150 μL DMSO. Formazan crystals were dissolved by shaking the plate on a rocker. Absorbance was read in a microplate reader (Infinite M Nano, Tecan) at a wavelength of at 570nm.

Production of TNF α , IL-6, IL-1 β , MCP-1, and RANTES

BV-2 microglia were seeded out in a 24-well plate at 5×10^5 cells/ml and treated with 25 and 50 $\mu\text{g/ml}$ of freeze-dried plant infusions for 30 min, followed by stimulation with HZ (400 $\mu\text{g/ml}$) for 24 h. At the end of the stimulation period, culture supernatants were collected. Levels of TNF α , IL-6, and IL-1 β in culture supernatants were measured using mouse ELISA assay kits (Biolegend). Production of CCL2 was evaluated using Invitrogen™ eBioscience™ Mouse CCL2 (MCP-1) ELISA Ready-SET-Go!™ kit (Thermo Scientific), while CCL5 production was measured with Mouse CCL5/RANTES DuoSet ELISA (R and D Systems). All ELISA

measurements were carried out according to the manufacturer's instructions and absorbance measured at 450nm in a Tecan Infinite M Nano microplate reader.

Nitrite production

BV-2 microglia were seeded out in a 24-well plate at 5×10^5 cells/ml and treated with 25 and 50 $\mu\text{g/ml}$ of plant infusions for 30 min, followed by stimulation with HZ (400 $\mu\text{g/ml}$) for 24 h. At the end of the stimulation period, culture supernatants were collected, and levels of NO were determined using the Griess assay kit (Promega).

Caspase-Glo[®]1 inflammasome assay

The caspase-Glo[®]1 inflammasome assay (Promega) was used to evaluate the effect of preparations on caspase-1 activity in BV-2 microglia stimulated with hemozoin. BV-2 cells were seeded in 96-well plates and pre-treated with 25 and 50 $\mu\text{g/ml}$ of plant infusions 30 min prior to activation with HZ (400 $\mu\text{g/ml}$) for a further 24 h. Activity of caspase-1 in cells was thereafter measured according to the manufacturer's instructions. Luminescence was read with FLUOstar OPTIM reader (BMG LABTECH).

In cell western assays for iNOS and NLRP3 proteins

BV-2 microglia were seeded into 96-well plates and treated with 25 and 50 $\mu\text{g/ml}$ of plant infusions and incubated for 30 min prior to stimulation with HZ (400 $\mu\text{g/ml}$) for a further 24 h. At the end of each experiment, cells were fixed with 8% paraformaldehyde solution (100 μL) for 15 min., followed by washing with PBS. The cells were then incubated with rabbit anti-iNOS (Abcam) and rabbit anti-NLRP3 (Abcam) antibodies overnight at 4°C. Thereafter, cells were washed with PBS and incubated with anti-rabbit HRP secondary antibody for 2 h at room temperature. Then, 100 μL avidin HRP substrate was added to each well and absorbance

measured at 450nm with a Tecan Infinite M Nano microplate reader. Readings were normalised with Janus Green normalisation stain (Abcam).

NF- κ B transcription factor assay

Effects of plant infusions on DNA binding of NF- κ B following stimulation by hemozoin was quantitatively evaluated using the NF- κ B transcription factor assay kit (Abcam). Following treatment of BV-2 microglia with 25 and 50 μ g/ml of plant infusions for 30 min, the cells were stimulated with HZ (400 μ g/ml) for 60 min. This was followed by preparation of nuclear extracts, which were then used in DNA binding assays according to the manufacturer's instructions. Absorbance was measured at 450nm with a Tecan Infinite M Nano microplate reader.

Phospho-p65 NF- κ B and phospho-I κ B α ELISAs

BV-2 microglia cells were seeded out into 6-well plates and treated with 25 and 50 μ g/ml of freeze-dried plant infusions and incubated for 30 min prior to stimulation with HZ (400 μ g/ml) for a further 60 min. At the end of the experiments, plates were washed with PBS, followed by the addition of cell lysis buffer (Cell Signalling Technology). The plate was then incubated on ice for 5 min and cells scraped. Extracts were sonicated and centrifuged at 14,000 \times g at 4°C for 10 min. Protein levels of phospho-p65 in the resulting cell lysates were measured using PathScan® phospho-NF- κ B p65 (Ser536) sandwich ELISA kit. Absorbance (protein expression) was measured at 450nm with a Tecan Infinite M Nano microplate reader.

Statistical analysis

Data are expressed as mean \pm SEM for at least 3 independent experiments (n=3) and analysed using one-way analysis of variance (ANOVA) with post hoc Dunnett's

multiple comparison test. Statistical analysis were conducted using the GraphPad Prism software.

Results

Ethnopharmacological field surveys

Ethnopharmacological field surveys conducted in 5 locations within southwest Nigeria revealed that 12 of the plant species mentioned by traditional medicine practitioners had >50% fidelity level for treating symptoms of malaria and cerebral malaria (Table 1). Preliminary pharmacological evaluation of 25 and 50 µg/mL of freeze-dried plant infusions showed that *Alstonia boonei*, *Anacardium occidentale*, *Enantia chlorantha*, *Khaya senegalensis*, *Mangifera indica*, and *Nauclea latifolia* produced significant ($p < 0.05$) reduction in the increased production of TNF α in HZ-stimulated BV-2 microglia (Supplementary Data 1). Results of cell viability experiments showed that 25 and 50 µg/mL of freeze-dried infusions did not reduce viability of HZ-stimulated BV-2 microglia (Supplementary Data 2). These plants were then selected for further pharmacological evaluation.

Effects of freeze-dried plant infusions on the production of pro-inflammatory cytokines

Results in Figure 1A show that in response to stimulation with synthetic hemozoin (400 µg/mL), secretion of TNF α in BV-2 microglia was significantly ($p < 0.001$) increased. However, significant ($p < 0.001$) reduction in TNF α production was observed following pre-treatment with 25 and 50 µg/mL of *A. boonei*, *A. occidentale*, *E. chlorantha*, *K. senegalensis*, *M. indica*, and *N. latifolia* prior to stimulation with HZ. Similar reduction in HZ-induced increased production of IL-6 (Figure 1B) and IL-1 β (Figure 1C) were observed following pre-treatment of BV-2 microglia with 25 and

50 µg/mL of *A. boonei*, *A. occidentale*, *E. chlorantha*, *K. senegalensis*, *M. indica*, and *N. latifolia*.

Effects of freeze-dried plant infusions on the production of pro-inflammatory chemokines

Experiments to determine effects of plants on inflammatory chemokines showed that stimulation of BV-2 microglia with HZ (400 µg/mL) resulted in significant ($p < 0.001$) elevation in the release of MCP-1 (Figure 2A) and CCL-5/RANTES (Figure 2B).

When cells were pre-treated with freeze-dried *A. boonei* (25 and 50 µg/mL), MCP-1 production was reduced by 32.7% and 44.5%, respectively when compared with HZ stimulation alone. In the presence of 50 µg/mL of freeze-dried *A. occidentale*, *A. indica*, *E. chlorantha*, *K. senegalensis*, *M. indica*, and *Nauclea latifolia*, HZ-induced increased production of MCP-1 was reduced by 47.1%, 47.2%, 42%, 38.6%, 48.5% and 38.9%, respectively (Figure 2A). Similarly, pre-treatment of HZ-stimulated BV-2 microglia with 50 µg/mL of freeze-dried *A. boonei*, *A. occidentale*, *A. indica*, *E. chlorantha*, *K. senegalensis*, *M. indica*, and *Nauclea latifolia* resulted in the reduction of RANTES by 54.2%, 48.5%, 50.3%, 39.2%, 40.9%, 47.5%, and 38.5, respectively (Figure 2B).

Freeze-dried plant infusions inhibited iNOS/NO production in hemozoin-stimulated BV-2 microglia

Further experiments to evaluate effects of freeze-dried plants on HZ-induced increased production of inflammatory mediators in BV-2 microglia revealed marked reduction in NO production by freeze-dried *A. boonei* (43.9% reduction with 50 µg/mL), *A. occidentale* (49.3% reduction with 50 µg/mL), *A. indica* (41.4% reduction with 50 µg/mL), and *M. indica* (47.5% reduction with 50 µg/mL)

(Figure 3A). A similar trend was observed in results obtained from in cell western experiments to determine effects of freeze-dried plants on HZ-induced increased expression of iNOS protein in BV-2 microglia. As shown in Figure 3B, pre-treatment of BV-2 microglia with 50 µg/mL of *A. boonei*, *A. occidentale*, *A. indica*, and *M. indica* resulted in 48.9%, 45.8%, 53.1% and 51.2% reduction in iNOS protein levels, respectively. Modest reductions in HZ-induced elevated NO production and iNOS protein expression were achieved with *E. chlorantha*, *K. senegalensis* and *N. latifolia* (Figures 3A & 3B).

Anti-inflammatory effects of freeze-dried plants infusions were mediated through mechanisms involving NF-κB activation

Encouraged by results showing varying degrees of anti-inflammatory activity by the plants, we then investigated their effects on HZ-induced activation of the NF-κB transcription factor. Interestingly, we observed that incubating BV-2 microglia with 50 µg/mL of *A. boonei*, *A. indica*, *A. occidentale*, *E. chlorantha* and *M. indica* prior to stimulation with HZ (400 µg/mL) produced >40% reduction in the levels of phospho-p65 protein measured using ELISA (Figure 4A).

We further used a transcription factor assay to evaluate the impact of pre-treatment with freeze-dried plants on nuclear DNA binding of NF-κB following stimulation of BV-2 microglia with HZ (400 µg/mL). Results in Figure 4B shows that 50 µg/mL of freeze-dried *A. boonei*, *A. occidentale*, *A. indica*, *M. indica* and *N. latifolia* inhibited HZ-induced increased DNA binding by 50.5%, 48.8%, 53.4%, 49.6%, and 43.4%, respectively (Figure 4B).

Freeze-dried plant infusions reduced NLRP3 inflammasome protein and caspase-1 activity in hemozoin-stimulated BV-2 microglia

Hemozoin has been reported to induce the release of IL-1 β through mechanisms involving activation of NLRP3 inflammasome/caspase-1 activity in BV-2 microglia [9].

Following results showing inhibition of HZ-induced increased IL-1 β production by freeze-dried plants, we investigated their effects of protein levels of NLRP3 inflammasome, as well as caspase-1 activity in HZ-stimulated BV-2 microglia.

Results in Figure 5A show that increased protein expression of NLRP3 inflammasome following stimulation with HZ (400 μ g/mL) was significantly reduced ($p < 0.05$) in the presence of 25 and 50 μ g/mL of all freeze-dried plant preparations. Pre-treatment with *A. indica* (50 μ g/mL) produced the highest inhibition (58.6%), while *A. occidentale* (50 μ g/mL), *M. indica* (50 μ g/mL) and *A. boonei* (50 μ g/mL) inhibited NLRP3 expression by 54.1%, 49.2% and 47.1%, respectively.

Interestingly, HZ-induced increased BV-2 microglia caspase-1 activity was significantly reduced ($p < 0.05$) by both 25 and 50 μ g/mL of *A. boonei*, *A. occidentale*, *A. indica*, *E. chlorantha*, and *M. indica*. However, significant ($p < 0.05$) reduction in activity was achieved with 50 μ g/mL of *K. senegalensis*, while *N. latifolia* did not produce significant reduction in activity at both concentrations investigated (Figure 5B).

Discussion

Some medicinal plants used in the treatment of symptoms of malaria have been proven to provide symptomatic relief because of their anti-inflammatory activity in reducing levels of mediators which contribute to fever, aches and pains. Similarly, it has been suggested that some of these plants may be valuable in the adjunctive

treatment of neurological sequelae of cerebral malaria due to their anti-inflammatory activity.

Using the Fidelity Level (FL) as a measure of consensus on the use of plants for treating symptoms of malaria and/or cerebral malaria, *Ageratum conyzoides* (leaves), *Alstonia boonei* (stem bark), *Anacardium occidentale* (stem bark), *Azadiractha indica* (leaves), *Chromolaena odorata* (whole plant), *Enantia chlorantha* (stem bark), *Khaya senegalensis* (stem bark), *Mangifera indica* (stem bark), *Nauclea latifolia* (stem bark), *Psidium guajava* (leaves), *Tithonia diversifolia* (leaves) and *Vernonia amygdalina* (leaves) were reported to score an FL greater than 50%. Interestingly, studies have demonstrated antimalarial activities of some of these plants *in vitro* and *in vivo* (Awe et al. 1998; Benoit-Vical et al. 1998; Elufioye and Agbedahunsi 2004; Udeinya et al. 2006; Nour et al. 2010; Owuor et al. 2012; Olanlokun et al. 2012; Kemgne et al. 2012; Ndjonka et al. 2012; Adepiti et al. 2014; Ezenyi et al. 2014; Bihonegn et al. 2019).

In preliminary pharmacological investigations on freeze-dried infusions of the plants, *Alstonia boonei*, *Anacardium occidentale*, *Azadiractha indica*, *Enantia chlorantha*, *Khaya senegalensis*, *Mangifera indica*, and *Nauclea latifolia* produced significant reduction of increased production of TNF α in BV-2 microglia stimulated with a synthetic hemozoin. Further investigations revealed that these plants reduced the production of the pro-inflammatory cytokines IL-6, IL-1 β as well as chemokines MCP-1 and CCL-5/RANTES in BV-2 microglia stimulated with hemozoin.

The effects of these plants in reducing the production of pro-inflammatory cytokines and chemokines is remarkable considering the roles of these mediators in the pathogenesis of cerebral malaria. Increased levels of TNF α , IL-6 and IL-1 β have been reported to be associated with the pathogenesis of cerebral malaria (Idro et al.

2010). Also, studies have shown that chemokines such as MCP-1 and RANTES/CCL5 contribute to the pathogenesis of this malaria complication (Pacher et al. 2007). Furthermore, we have shown that a synthetic form of the malaria metabolic product hemozoin increased pro-inflammatory cytokine production in BV-2 microglia (Velagapudi et al. 2019), confirming their roles in CNS immune responses to malaria infection.

Excessive production of nitric oxide (NO) by brain microglia during neuroinflammation has been linked to damage to adjacent neurons. In cerebral malaria, iNOS-mediated NO production has been postulated to be cytotoxic to neurons through different mechanisms (Pacher et al. 2007). Results from this study show strong inhibition of iNOS-mediated increased production of NO by freeze-dried infusions of *Alstonia boonei*, *Anacardium occidentale*, *Azadiractha indica*, and *Mangifera indica* in hemozoin-stimulated BV-2 microglia, while *Enantia chlorantha*, *Khaya senegalensis* and *Nauclea latifolia* produced modest effects. These results further demonstrate the potential benefits of these plants as herbal remedies in the treatment of cerebral malaria, at least due to their ability to reduce inflammatory responses in the brain. Previous studies have reported anti-inflammatory effects for *Alstonia boonei* (Olajide et al. 2000), *Anacardium occidentale* (Olajide et al. 2004), *Azadiractha indica* (Okpanyi and Ezeukwu 1981), and *Mangifera indica* (Ojewole 2005), which may explain their strong anti-inflammatory activity against hemozoin-induced neuroinflammation observed in this study.

Activation of the NF- κ B is known to regulate the production of pro-inflammatory cytokines, chemokines and mediators such nitric oxide. Activation of this transcription factor has also been implicated in the pathology of cerebral malaria (Kumar et al. 2003; Tripathi et al. 2006; Tripathi et al. 2009), and has been shown to

be one of the critical mechanisms involved in hemozoin-induced neuroinflammation (Velagapudi et al. 2019). These links, coupled with our results showing marked reduction in the production of inflammatory mediators by plants under investigation encouraged us to investigate their effects on the activation of NF- κ B following stimulation of BV-2 microglia with synthetic hemozoin. Consistent with their effects on TNF α , IL-6, IL-1 β , MCP-1, RANTES/CCL5, and INOS/NO, freeze-dried infusions of *Alstonia boonei*, *Anacardium occidentale*, *Azadiractha indica*, and *Mangifera indica* produced significant inhibition of hemozoin-induced increased phosphorylation of NF- κ B p65 sub-unit and its binding to the DNA in the nucleus, an outcome suggesting that the plants may be reducing inflammation through mechanisms involving NF- κ B activation. Studies have previously shown that an extract of *Anacardium occidentale* inhibited neuroinflammation in lipopolysaccharide-stimulated BV-2 microglia, through mechanisms involving NF- κ B (Olajide et al. 2013). Similarly, modulation of NF- κ B activation has been suggested as a possible mechanism for the anti-inflammatory activity of *Azadiractha indica* (Pooladanda et al. 2019) and *Mangifera indica* (Garrido et al. 2005).

There have been suggestions in scientific literature that malaria hemozoin activates NLRP3 inflammasome/caspase-1 pathway to release IL-1 β (Dostert et al. 2009; Shio et al. 2009; Velagapudi et al. 2019; Ogunrinade et al. 2021). In this study, we have demonstrated significant inhibition of hemozoin-induced NLRP3 protein expression by all plants investigated. However, *Khaya senegalensis* and *Nauclea latifolia* did not have effect on hemozoin-induced caspase-1 activity.

Results of this study have shown that freeze-dried samples of *Alstonia boonei*, *Anacardium occidentale*, *Azadiractha indica* and *Mangifera indica* produced strong

activity in reducing synthetic hemozoin-induced neuroinflammation in BV-2 microglia through mechanisms involving NF- κ B and NLRP3 inflammasome activation.

Moderate activities have been shown by *Enantia chlorantha*, *Khaya senegalensis* and *Nauclea latifolia*. The outcome of this study provides pharmacological evidence for the use of these plants in Traditional African Medicine for treating symptoms and complications of malaria, including cerebral malaria. It is not yet clear how these plants may be acting *in vivo* to overcome the blood-brain barrier, and should be investigated in human intervention studies involving patients presenting with malaria and/or cerebral malaria.

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Data Availability

All data used during this study are available from the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Table and Figure Legends

Table 1: Plants with FL>50% in the treatment of malaria and/or cerebral malaria by traditional medicine practitioners in some locations of southwest Nigeria

Figure 1: Effects of freeze-dried infusions of plants on increased production of TNF α (A), IL-6 (B), and IL-1 β (C) in HZ-stimulated BV-2 microglia. Cells were treated with 25 and 50 μ g/mL of freeze-dried infusions prior to stimulation with HZ (400 μ g/mL) for 24 h. Levels of cytokines were detected using mouse ELISA kits. Values are mean \pm SEM for at least 3 independent experiments (### $p < 0.001$ unstimulated control *versus* HZ stimulation. *** $p < 0.001$, treatments *versus* HZ stimulation; one-way ANOVA with post-hoc Dunnett's multiple comparison test).

Figure 2: Effects of freeze-dried infusions of plants on increased production of MCP-1 (A) and CCL-5/RANTES (B) in HZ-stimulated BV-2 microglia. Cells were treated with 25 and 50 μ g/mL of freeze-dried infusions prior to stimulation with HZ (400 μ g/mL) for 24 h. Levels of chemokines were detected using mouse ELISA kits. Values are mean \pm SEM for at least 3 independent experiments (### $p < 0.001$ unstimulated control *versus* HZ stimulation. *** $p < 0.001$, treatments *versus* HZ stimulation; one-way ANOVA with post-hoc Dunnett's multiple comparison test).

Figure 3: Effects of freeze-dried infusions of plants on increased production of NO (A) and iNOS protein expression (B) in HZ-stimulated BV-2 microglia. Cells were treated with 25 and 50 μ g/mL of freeze-dried infusions prior to stimulation with HZ (400 μ g/mL) for 24 h. Levels of NO were detected using the Griess assay, while iNOS protein expression was measured with in cell western ELISA. Values are mean \pm SEM for at least 3 independent experiments (### $p < 0.001$ unstimulated control *versus* HZ

stimulation. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, treatments *versus* HZ stimulation; one-way ANOVA with post-hoc Dunnett's multiple comparison test).

Figure 4: Effects of freeze-dried infusions of plants on increased phospho-p65 protein expression (A) and NF- κ B DNA binding (B) in HZ-stimulated BV-2 microglia. Cells were treated with 25 and 50 $\mu\text{g/mL}$ of freeze-dried infusions prior to stimulation with HZ (400 $\mu\text{g/mL}$) for 60 min. Values are mean \pm SEM for at least 3 independent experiments (### $p < 0.001$ unstimulated control *versus* HZ stimulation. *** $p < 0.001$, treatments *versus* HZ stimulation; one-way ANOVA with post-hoc Dunnett's multiple comparison test).

Figure 5: Effects of freeze-dried infusions of plants on increased NLRP3 protein expression (A) and caspase-1 activity (B) in HZ-stimulated BV-2 microglia. Cells were treated with 25 and 50 $\mu\text{g/mL}$ of freeze-dried infusions prior to stimulation with HZ (400 $\mu\text{g/mL}$) for 24 h. Values are mean \pm SEM for at least 3 independent experiments (### $p < 0.001$ unstimulated control *versus* HZ stimulation. ns (not significant); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, treatments *versus* HZ stimulation; one-way ANOVA with post-hoc Dunnett's multiple comparison test).

Table 1

Plant Name	Part used	FL (%)
<i>Ageratum conyzoides</i>	Leaves	67
<i>Alstonia boonei</i>	Stem bark	77
<i>Anacardium occidentale</i>	Stem bark	87
<i>Azadiractha indica</i>	Leaves	81
<i>Chromolaena odorata</i>	Whole plant	79
<i>Enantia chlorantha</i>	Stem bark	86
<i>Khaya senegalensis</i>	Stem bark	75
<i>Mangifera indica</i>	Stem bark	77
<i>Nauclea latifolia</i>	Stem bark	86
<i>Psidium guajava</i>	Leaves	78
<i>Tithonia diversifolia</i>	Leaves	89
<i>Vernonia amygdalina</i>	Leaves	75

Figure 1

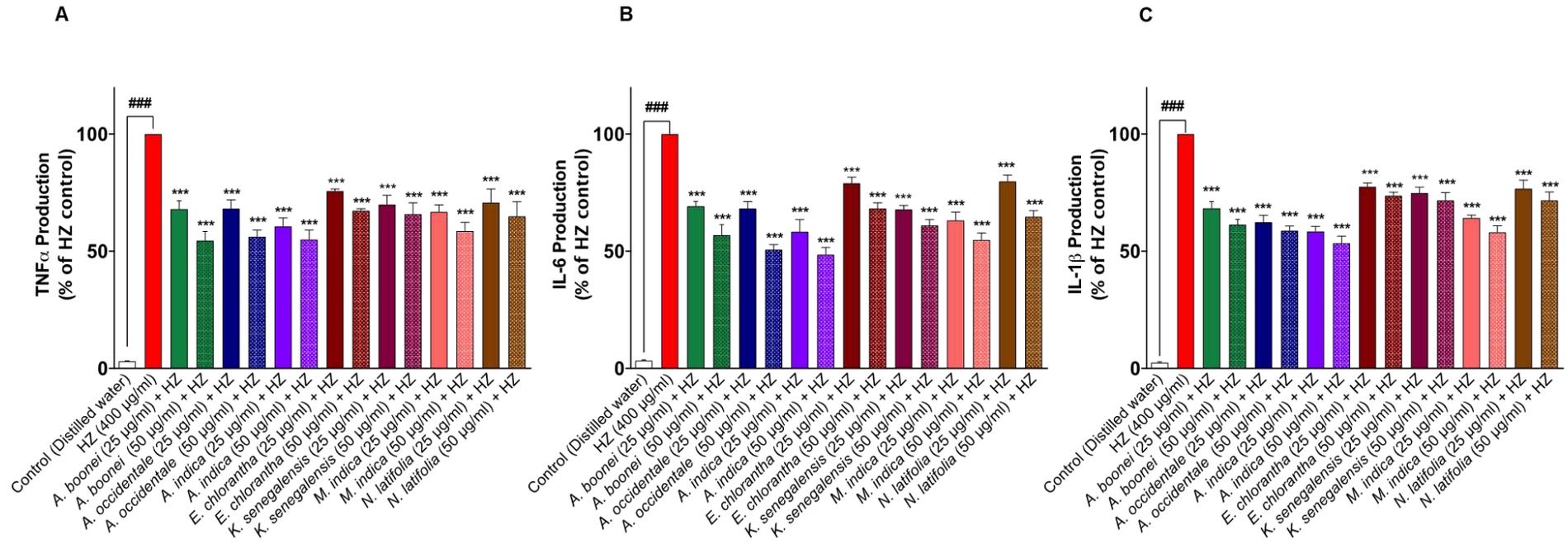
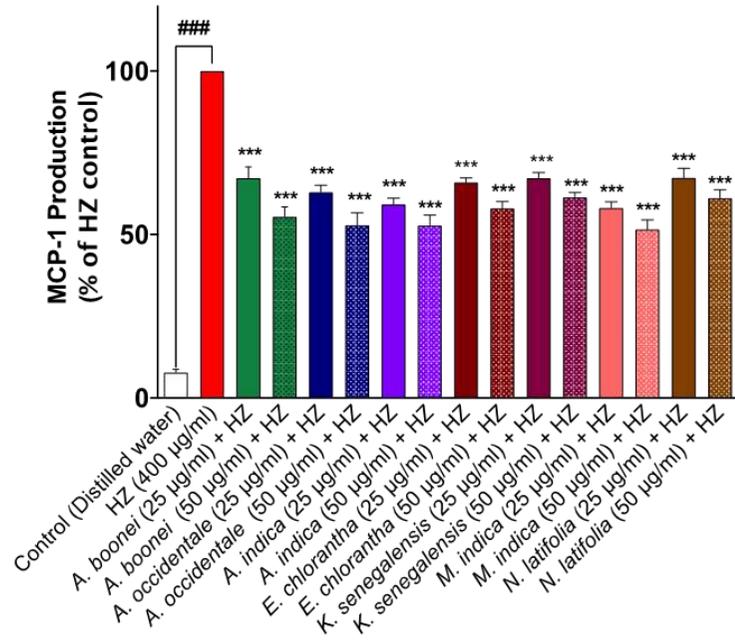


Figure 2

A



B

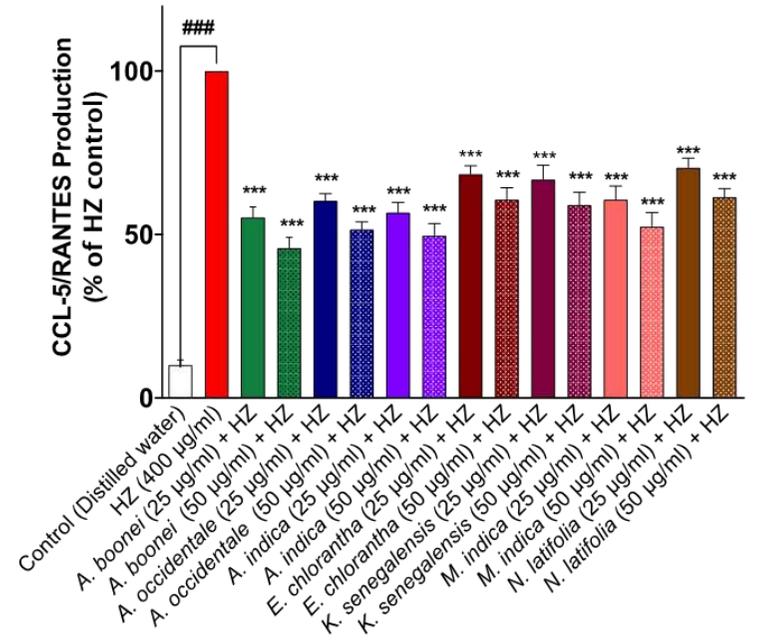
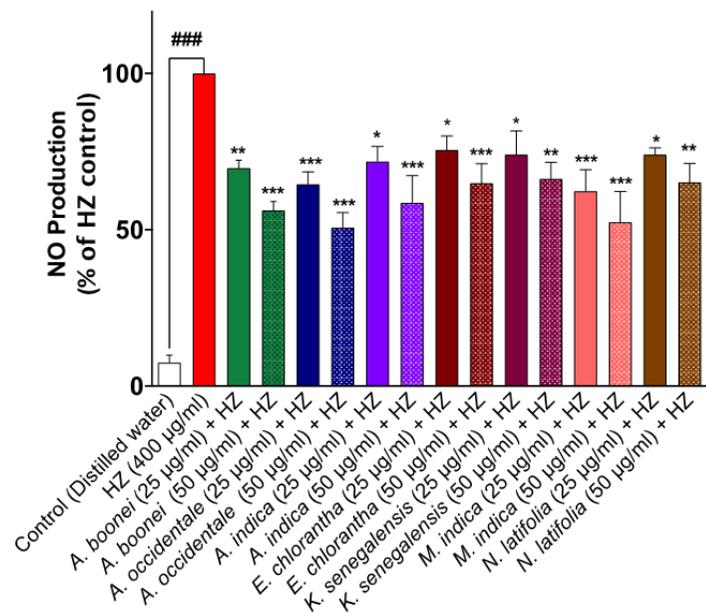


Figure 3

A



B

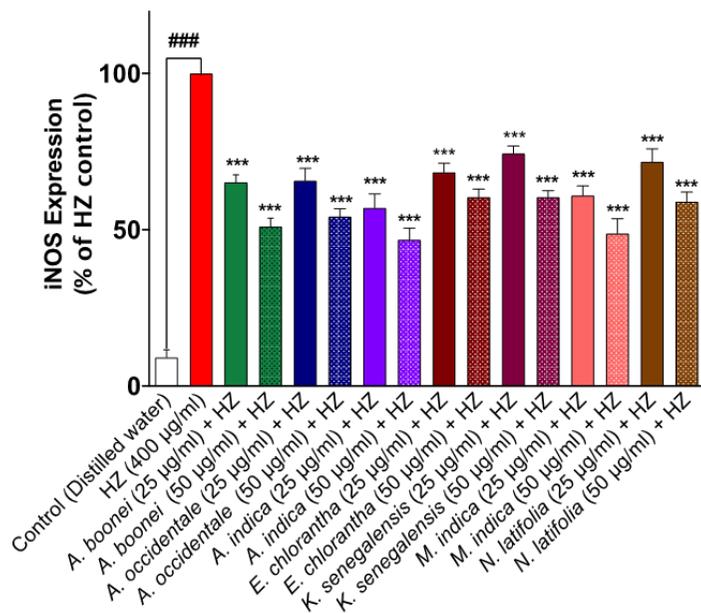
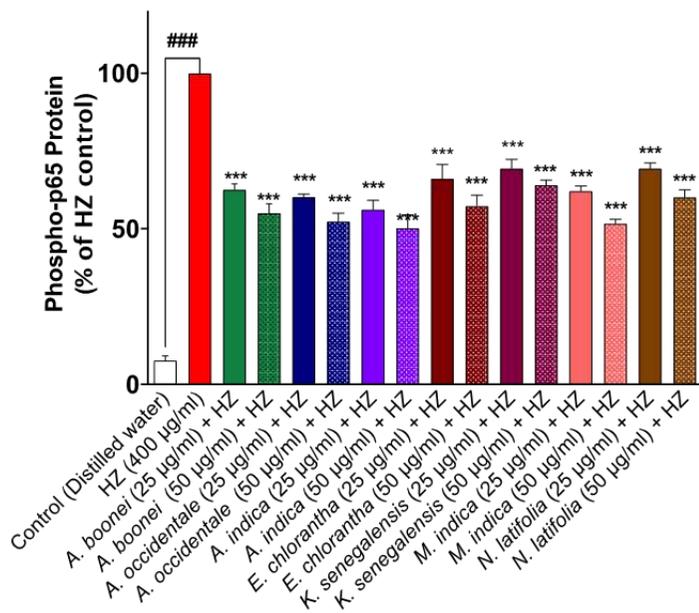


Figure 4

A



B

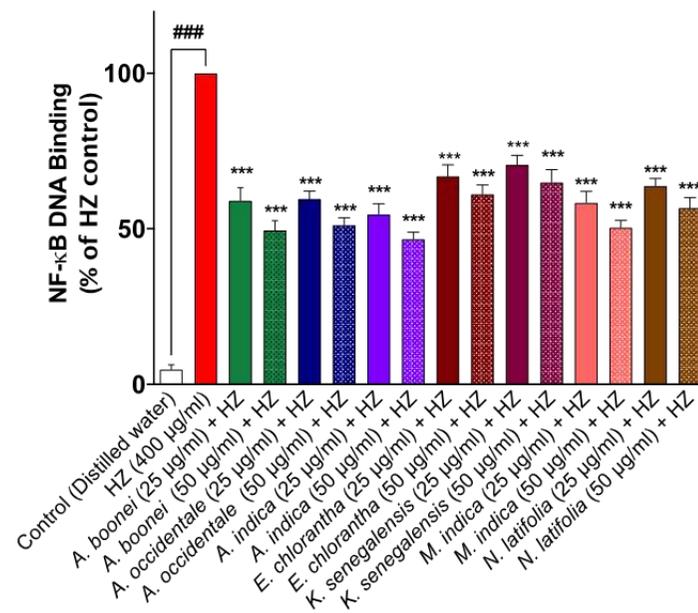
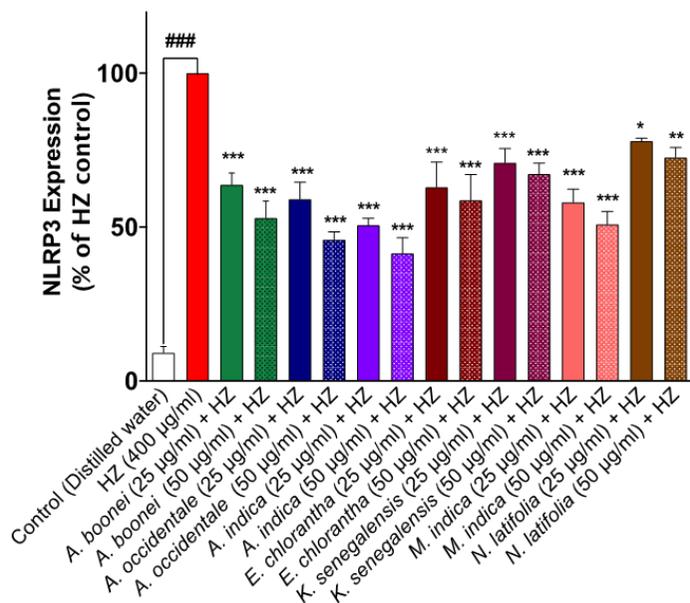
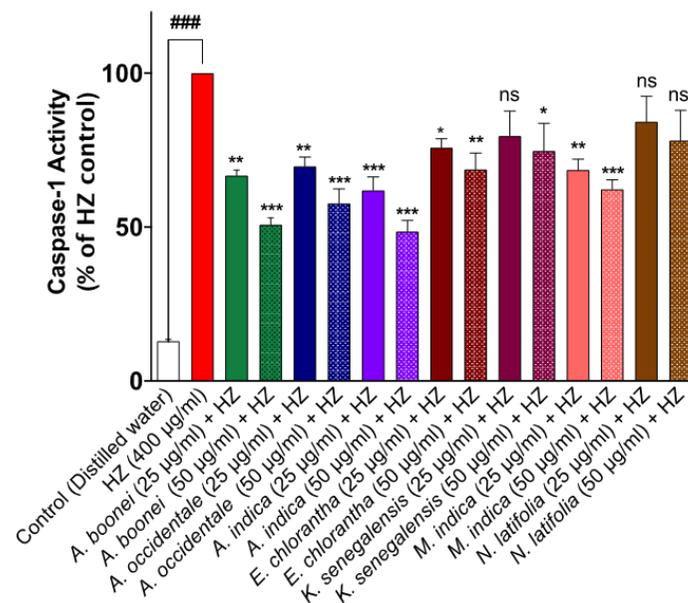


Figure 5

A



B



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