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Azadirachta indica ethanolic extract protects neurons from apoptosis and mitigates brain swelling in experimental cerebral malaria

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Abstract

Background: Cerebral malaria is a rapidly developing encephalopathy caused by the apicomplexan parasite *Plasmodium falciparum*. Drugs currently in use are associated with poor outcome in an increasing number of cases and new drugs are urgently needed. The potential of the medicinal plant *Azadirachta indica* (Neem) for the treatment of experimental cerebral malaria was evaluated in mice.

Methods: Experimental cerebral malaria was induced in mice by infection with *Plasmodium berghei* ANKA. Infected mice were administered with *Azadirachta indica* ethanolic extract at doses of 300, 500, or 1000 mg/kg intraperitoneally (i.p.) in experimental groups, or with the anti-malarial drugs chloroquine (12 mg/kg, i.p.) or artemether (1.6 mg/kg, i.p.), in the positive control groups. Treatment was initiated at the onset of signs of brain involvement and pursued for five days on a daily basis. Mice brains were dissected out and processed for the study of the effects of the extract on pyramidal cells' fate and on markers of neuroinflammation and apoptosis, in the medial temporal lobe.

Results: *Azadirachta indica* ethanolic extract mitigated neuroinflammation, decreased the severity of brain oedema, and protected pyramidal neurons from apoptosis, particularly at the highest dose used, comparable to chloroquine and artemether.

Conclusions: The present findings suggest that *Azadirachta indica* ethanolic extract has protective effects on neuronal populations in the inflamed central nervous system, and justify at least in part its use in African and Asian folk medicine and practices.

Keywords: Cerebral malaria, *Azadirachta indica* extract, Pyramidal neurons, Neuroinflammation, Brain oedema

Background

Cerebral malaria is a rapidly developing encephalopathy that represents the most severe neurological complication of infection with *Plasmodium falciparum*. Cerebral malaria mainly occurs in resource-poor tropical countries, and mostly affects children [1,2]. Surviving patients display neurologic and cognitive deficits, including consciousness impairment, cerebellar ataxia, seizures, and coma, making cerebral malaria a major cause of childhood neurodisability in endemic areas

[3-6]. The pathogenesis of neuro-cognitive sequelae is poorly understood.

Experimental models of cerebral malaria have been providing new mechanistic insights that will help develop efficient neuro-protective interventions. The non-human pathogenic parasite *Plasmodium berghei* has been used to induce experimental cerebral malaria in mice that has some features comparable to the human disease [7-9]. Experimental evidence suggests that the vast array of pro-inflammatory molecules released by the host to fight the infection and alterations due to parasite sequestration in the microvessels cause leakage of plasma into the parenchyma resulting in brain oedema, and may contribute to the subsequent blood-brain

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barrier breakdown [10,11]. Brain parenchyma infiltration by danger or pathogen-associated molecules would trigger a sustained detrimental neuroinflammatory response leading to the recruitment of circulating immune cells. The latter cells would induce death through cell-mediated apoptosis in sensible neuronal populations, by triggering pathways such as caspase 3 and Fas-Fas ligand [12-14]. Previous studies and reports have shown that such inflammation-triggered apoptosis can induce cerebellar ataxia when affecting Purkinje cells in the cerebellum [15-17]. Similarly, apoptosis affecting cortical neurons may explain many other neurologic deficits observed in experimental cerebral malaria, such as cognitive impairment or seizures [18-21]. Overall, these findings corroborate the alterations reported in humans [9,14,22,23]. New effective drugs are urgently needed for cerebral malaria treatment due to the development of parasite chemoresistance, and to improve current drugs' poor treatment outcome [24-26].

Azadirachta indica is a plant commonly used in the African and Asian folk medicine and practices [27-31]. Anti-malarial effects of *A. indica* extracts have been demonstrated [32-36], as well as the neuro-protective effects on Purkinje cells of *P. berghei*-infected mice successfully mitigating cerebellum malaria [16]. The present study addresses the potential of *A. indica* extract for the protection of cortical and sub-cortical pyramidal neurons of *P. berghei*-infected mice, considering the implications for the treatment of cerebral malaria-associated cortical deficits.

Methods

Experimental procedure

Thirty five mice, divided into seven groups (five animals per group), were used in this study. These groups were: *i*) a disease control group made of untreated *P. berghei*-infected mice; *ii*) two positive control groups made of *P. berghei*-infected mice treated with the commercially available anti-malarial drugs chloroquine (12 mg/kg in distilled water, i.p.) and artemether (1.6 mg/kg in olive oil, i.p.); *iii*) three experimental groups made of *P. berghei*-infected mice treated with *A. indica* ethanolic extract at doses 300, 500, or 1000 mg/kg (i.p.); *iv*) and a group of uninfected healthy animals. Parasitaemia, body weight, core body temperature, and signs of brain involvement were monitored. Treatment was started when the number of parasitized red blood cells was >5% (severe malaria indicator) and was associated with signs of brain involvement. Treatment was pursued for five days on a daily basis. Mice either died spontaneously or were sacrificed by ether inhalation-induced deep anesthesia once signs of disease terminal stage (hypothermia, ptosis, and convulsion) [37], were observed. Uninfected mice were sacrificed concomitantly with the last animals to check for disease terminal

stage signs. For all animals, brains were dissected out and processed for histopathological analysis.

Animals and infection

Male Swiss albino mice (weighing 26.5 ± 3.0 g, aged 6 ± 1 week) were donated by Sudan National Health Laboratory (Khartoum, Sudan). Mice were maintained at a 12:12 light/dark cycle and were given ad libitum access to food (standard diet) and water. For infection, mice were administered (i.p.) with a load of 10×10^6 parasitized red blood cells containing *P. berghei* ANKA. The parasitaemia was checked by blood smear Giemsa-staining. All procedures received approval from the Ethical Committee of the Institute of Endemic Diseases of the University of Khartoum and animals were handled according to institutional guidelines. *Plasmodium berghei* ANKA blood-stage cryostabilates were kindly donated by Dr. C.R. Pillai (National Institute of Malaria, New Delhi, India).

Preparation of the extract

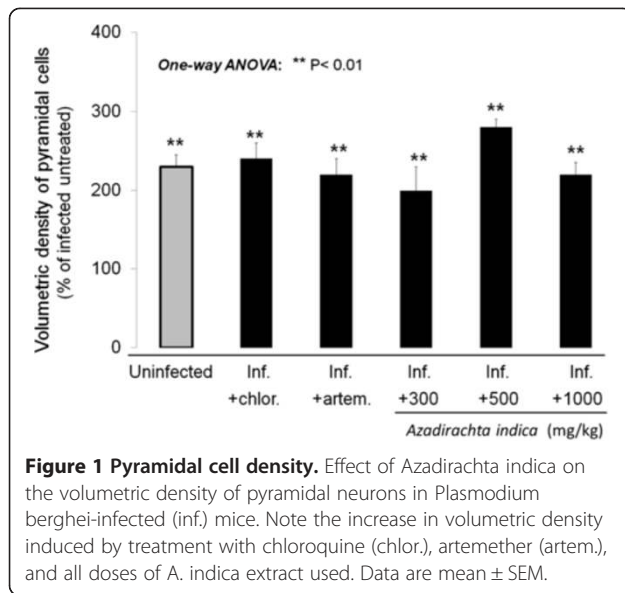
Fresh leaves of *A. indica* were collected in Khartoum region, where they are commonly used in folk medicine to treat malaria [33], and shade-dried. Dry leaves were grinded, and 1500 g of powder were macerated in 15 L of ethanol for three consecutive days at room temperature. A 1 mg/mL stock solution was obtained by filtration and boiling of the supernatant, and was stored at 4°C until use. Three doses of *A. indica*, i.e. 300, 500, and 1,000 mg/kg, were administered to the respective experimental groups once a day for five days. These regimens were chosen in accordance with previous pharmacological studies in malaria models [16,28].

Pyramidal neuron density and brain oedema

Brains dissected out were fixed in 10% neutral buffered formaldehyde, then paraffin-embedded and cut sagittally (section thickness: 5 μ m). Sections were deparaffinized in xylene, rehydrated, and processed for haematoxylin-eosin (H&E) staining. The pyramidal neuron volumetric density, i.e. the proportion of brain subfield that is occupied by pyramidal neuronal cell bodies, was determined as described by Highley and colleagues [38]. Pyramidal neurons were counted on five consecutive sections, in the medial temporal lobe, using a light microscope with a fixed 1 cm grid eyepiece under 40 \times objective. Brain oedema was assessed by giving a severity score integrating histopathological parameters, such as the importance (how big) and frequency (how numerous) of fluid built up in the brain parenchyma, on the H&E stained sections [39,40].

Immunohistochemistry

The sections processed for immunohistochemical labeling were deparaffinized in xylene, rehydrated, and endogenous

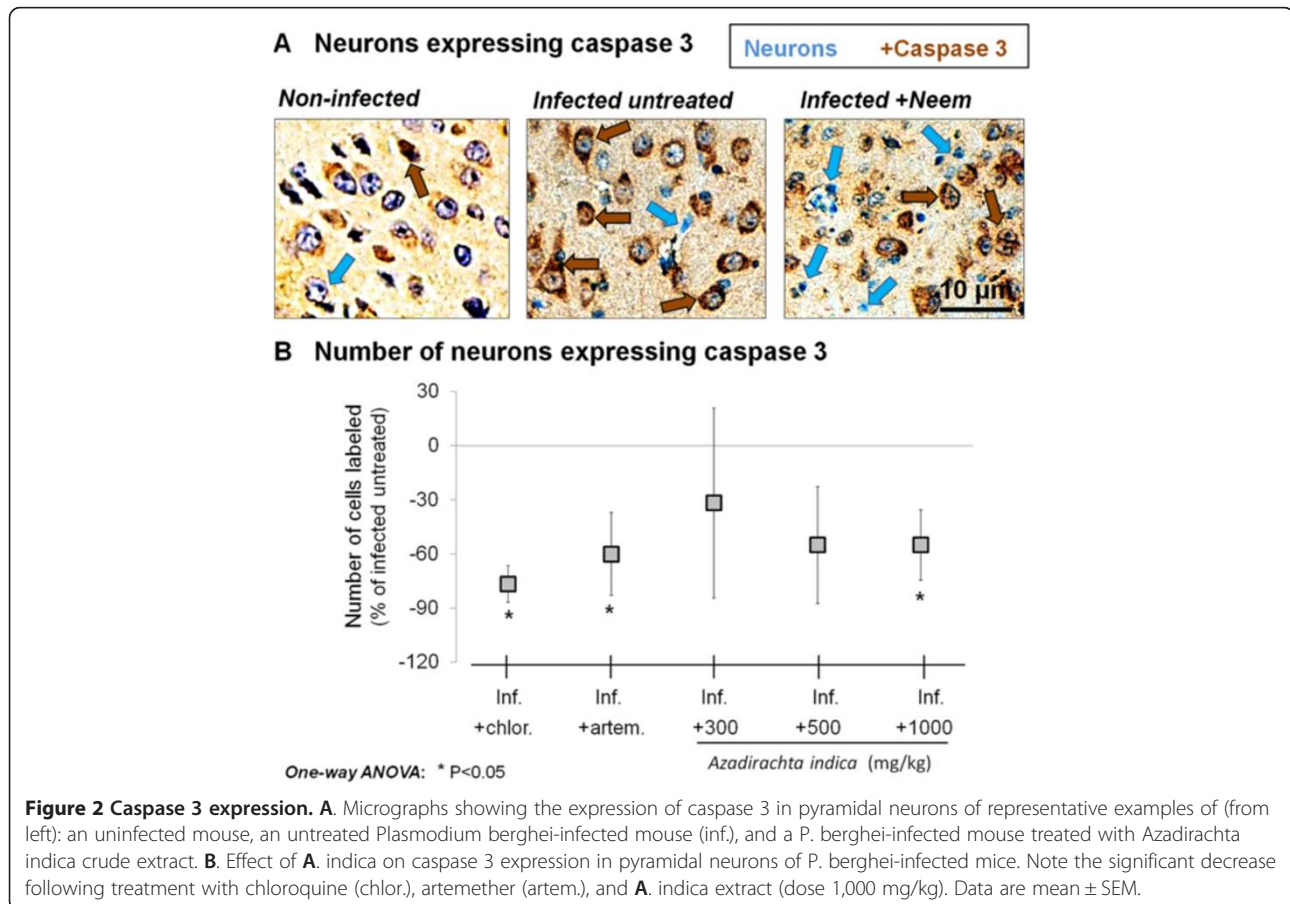


synthase (NOS) primary antibody buffer solution (dilution factor 1:40, Lica Biosystem Newcastle Ltd, UK), followed by a biotinylated secondary antibody and streptavidin-conjugated horseradish peroxidase (Vision Biosystems Novocastra, Novocastra Laboratories Ltd., Newcastle, UK) prepared according to the kit instructions. The sections were incubated with 3,3'-diaminobenzidine hydrochloride (DAB) chromogen substrate (Vision Biosystems Novocastra) according to the manufacturer's instructions, and counterstained with H&E. P value < .05 was considered significant. Thorough washes between steps were performed using immune wash buffer (Vision Biosystems Novocastra). Sections were dehydrated through a graded ethanol series, cleared in xylene, and covered with a thin glass coverslip. The fraction of pyramidal neurons expressing each of the studied markers, i.e. the markers of apoptosis caspase 3, Fas, and Fas ligand, and the markers of neuroinflammation-triggered apoptosis TNF and NOS, was determined using a light microscope under 40 \times objective.

peroxidase activity was extinguished. Sections were then pre-incubated in normal serum buffer solution (Diagnostic BioSystems, Serpentine, CA, USA), and incubated for 3 h in either rabbit anti-caspase-3, anti-FAS, anti-FAS ligand, anti-tumor necrosis factor (TNF), or anti-nitric oxide

Statistical analysis

Data obtained from infected treated or uninfected groups were compared to those obtained from the infected untreated group using one-way ANOVA followed by LSD



test. Differences with a p value < .05 were considered statistically significant. All data were expressed as a percentage of the value obtained in the infected untreated group. Data are presented as mean ± SEM (n = 5).

Results

General observations

The early signs of disease started five days after infection in the majority of *P. berghei*-infected animals, i.e. in the disease control group and in animals treated with chloroquine, artemether, or one of the three doses of *A. indica* extract. Cerebral malaria signs appeared at day 6 ± 1 post-infection. The most recurrent ones were a ruffled fur, locomotor impairments, and sickness behavior (anorexia, cachexia, fever) associated with body weight loss. Animals treated with *A. indica*, chloroquine or

artemether did not displayed fever (as evaluated by increases in core body temperature), and from dose 500 mg/kg of extract locomotor disturbances and body weight loss were improved. Parasitaemia was decreased in infected mice following treatment with chloroquine or arthemether (from 6.1 ± 1.8% to 1.2 ± 0.4%, P < 0.05), but no significant change was observed in *A. indica*-treated animals, as previously reported [16].

Azadirachta indica extract did not protected infected mice from death, unlike chloroquine and artemether, although the highest doses slightly (not significantly) delayed death time. Infected untreated mice spontaneously died at day 9 ± 1 post-infection, *A. indica*-treated mice treated with the highest doses started to die at day 11, whereas no animal died in groups treated with chloroquine and artemether during the time of observation.

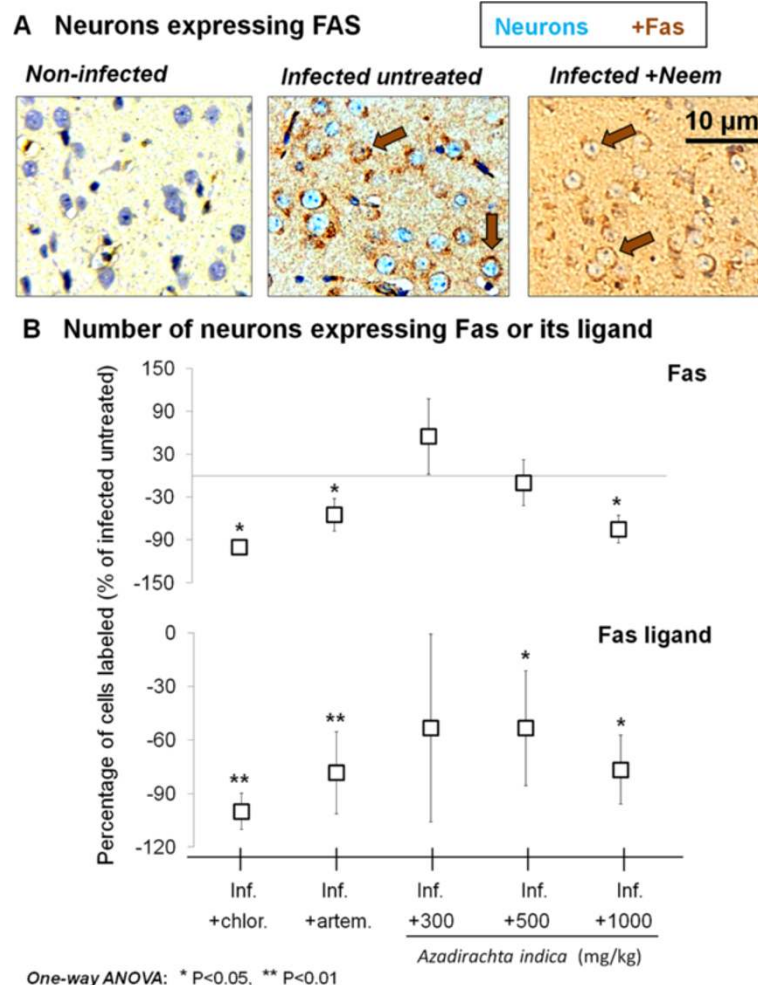


Figure 3 Fas and Fas ligand expression. **A.** Micrographs showing the expression of Fas in pyramidal neurons of representative examples of (from left): an uninfected mouse, an untreated *Plasmodium berghei*-infected mouse (inf.), and a *P. berghei*-infected mouse treated with *Azadirachta indica* crude extract. **B.** Effect of *A. indica* on the expression of Fas (upper) and Fas ligand (lower) in pyramidal neurons of *P. berghei*-infected mice. Note the significant decrease in expression of both molecules following treatment with chloroquine (chlor.), artemether (artem.), and *A. indica* extract (more marked at dose 1,000 mg/kg). Data are mean ± SEM.

However, about all the survivors in groups treated with the extract were sacrificed two days after (day 13 post-infection) as terminal signs of cerebral malaria, i.e. hypothermia, ptosis, ataxia, and convulsion, were observed. All other animals, including those treated with chloroquine or artemether as well as uninfected ones were also sacrificed then.

Extract effect on pyramidal neuron density

The effect of *A. indica* on *P. berghei*-infected mice pyramidal neuron volumetric density is shown in Figure 1. The volumetric density of pyramidal neurons of mice treated with *A. indica* extract was significantly higher at all doses, in comparison to the infected untreated group ($P < 0.01$), and was comparable (non-significantly different [41]) to the values observed in the infected groups treated with chloroquine or artemether, and to uninfected group values, which were also significantly different from infected untreated group values ($P < 0.01$ for all three groups).

Extract effect on caspase 3 expression

The effect of *A. indica* on the expression of caspase 3 in pyramidal neurons of *P. berghei*-infected mice is shown in Figure 2. Pyramidal neurons of mice treated with

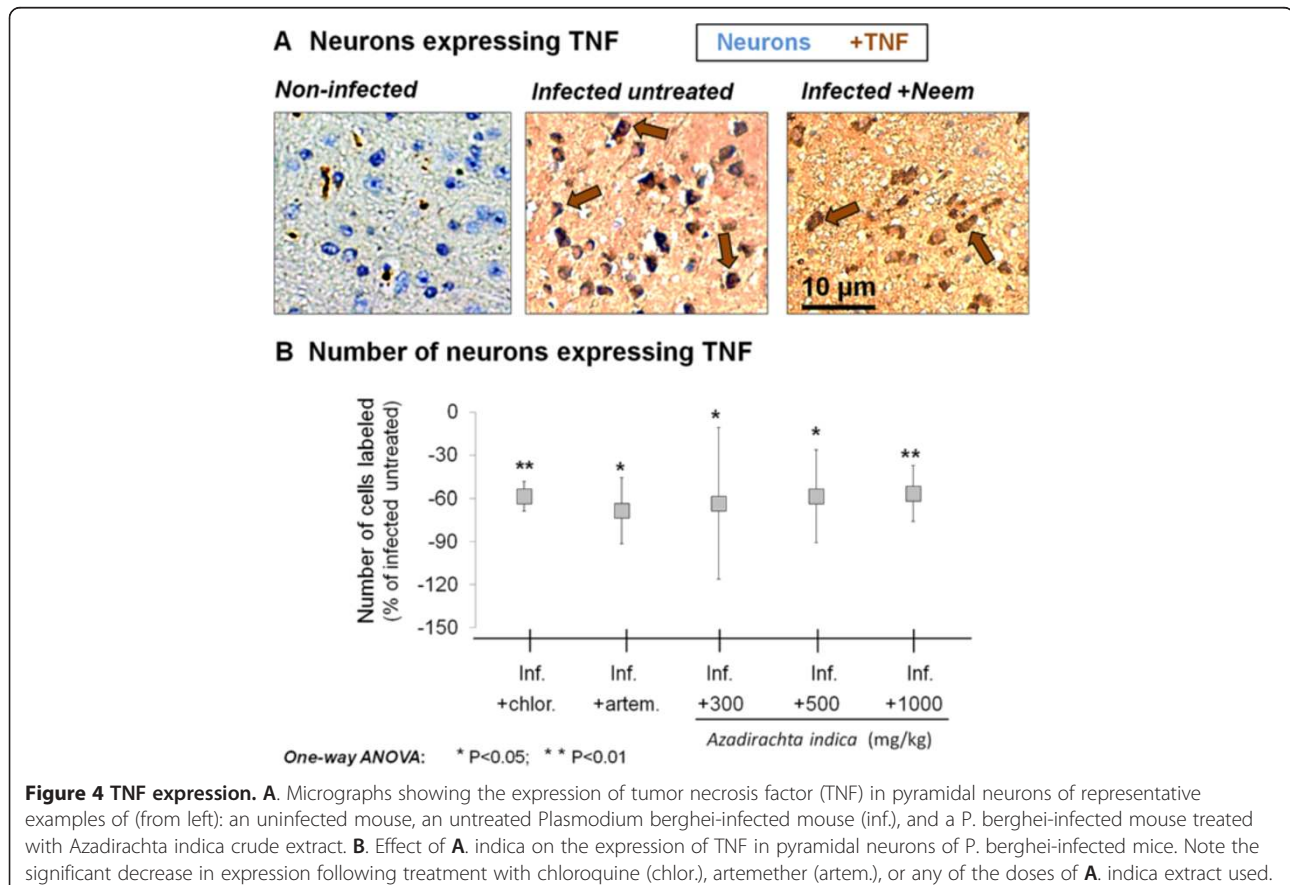
A. indica extract displayed a significant decrease in caspase 3 expression at the highest dose, in comparison to the infected untreated group ($P < 0.05$), comparable to the decreases induced by chloroquine ($P < 0.05$ vs. infected untreated group) or artemether ($P < 0.05$ vs. infected untreated group).

Extract effect on Fas and Fas ligand expression

The effect of *A. indica* on Fas and Fas ligand expression in *P. berghei*-infected mice pyramidal neurons is shown in Figure 3. Pyramidal neurons of mice treated with *A. indica* extract displayed a significant decrease in Fas expression at the highest dose, in comparison to the infected untreated group ($P < 0.05$) and a decrease in Fas ligand expression at doses 500 and 1000 mg/kg ($P < 0.05$ vs. infected untreated group). The significant decreases observed were comparable to those induced by chloroquine ($P < 0.05$ for Fas and $P < 0.01$ for Fas ligand vs. infected untreated group) or artemether ($P < 0.05$ for Fas and $P < 0.01$ for Fas ligand vs. infected untreated group).

Extract effect on TNF expression

The effect of *A. indica* on TNF expression in pyramidal neurons of *P. berghei*-infected mice is shown in Figure 4.



Pyramidal neurons of mice treated with *A. indica* extract displayed a significant decrease in TNF expression at all doses used, in comparison to the infected untreated group ($P < 0.01$ at 1000 mg/kg). This effect was comparable to the decrease in TNF expression induced by chloroquine ($P < 0.01$ vs. infected untreated group) or artemether ($P < 0.05$ vs. infected untreated group).

Extract effect on NOS expression

The effect of *A. indica* on the expression of NOS in *P. berghei*-infected mice pyramidal neuron is shown in Figure 5. *Azadirachta indica* extract induced a significant decrease in NOS expression, in comparison to the infected untreated group. However, such decrease was statistically significant and comparable to the effect of chloroquine ($P < 0.05$ vs. infected untreated group) or artemether ($P < 0.05$ vs. infected untreated group) only at the highest dose used (1000 mg/kg, $P < 0.05$).

Extract effect on brain oedema severity

The effect of *A. indica* extract on brain oedema severity in *P. berghei*-infected mice is shown in Figure 6. *Azadirachta indica* extract induced a significant decrease in brain oedema severity at all doses used, in comparison to the infected untreated group ($P < 0.05$), which was comparable

to the effect of chloroquine ($P < 0.01$ vs. infected untreated group) or artemether ($P < 0.05$ vs. infected untreated group).

Discussion

The present study points out the protective effects of *A. indica* extract on pyramidal neurons in experimental cerebral malaria induced by *P. berghei*-infection in mice.

Protective effects on pyramidal neurons

Pyramidal neuron density study revealed comparable values between *P. berghei*-infected mice treated with the extract of *A. indica* and uninfected mice, which were significantly different from untreated *P. berghei*-infected mice (disease control group) where a significant decrease in pyramidal neuron density, characteristic of cerebral malaria insult [14,18-20], was observed. These findings suggest that the extract protected pyramidal neurons from cerebral malaria-induced apoptosis. In order to further characterize these effects, the expression of apoptosis markers was studied, considering the importance of this phenomenon in cerebral malaria-associated encephalopathy [7-9].

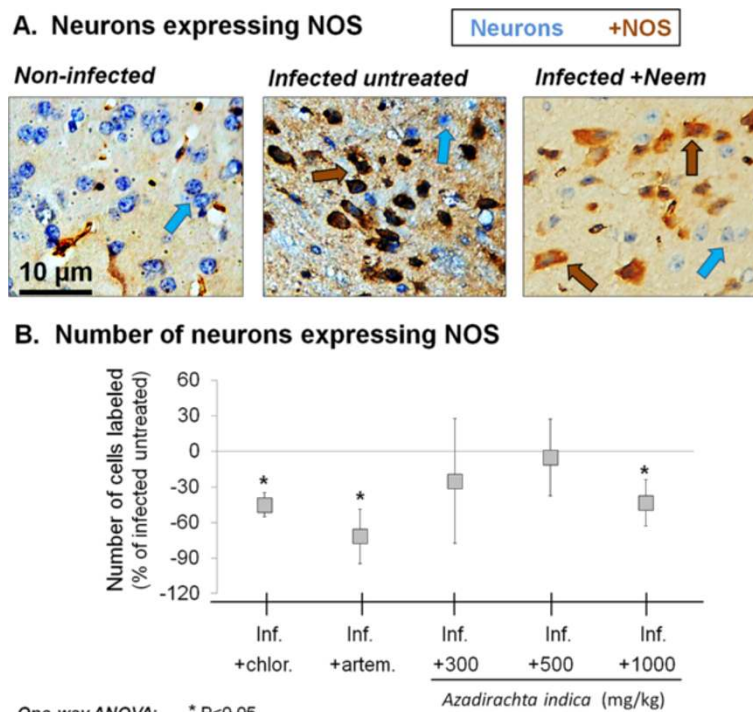
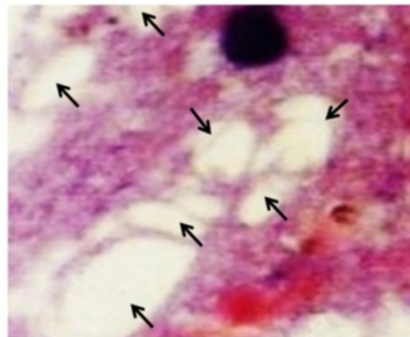


Figure 5 NOS expression. **A.** Micrographs showing the expression of nitric oxide synthase (NOS) in pyramidal neurons of representative examples of (from left): an uninfected mouse, an untreated Plasmodium berghei-infected mouse (inf.), and a *P. berghei*-infected mouse treated with *Azadirachta indica* crude extract. **B.** Effect of *A. indica* on the expression of NOS in pyramidal neurons of *P. berghei*-infected mice. Note the significant decrease in expression following treatment with chloroquine, artemether, and *A. indica* extract highest dose. Data are mean \pm SEM.

A Representative case with the highest brain edema severity score



A severity score of brain edema was given on the basis of the importance and frequency of fluid buildup in the brain parenchyma

↖ Fluid buildup

B Severity of brain edema

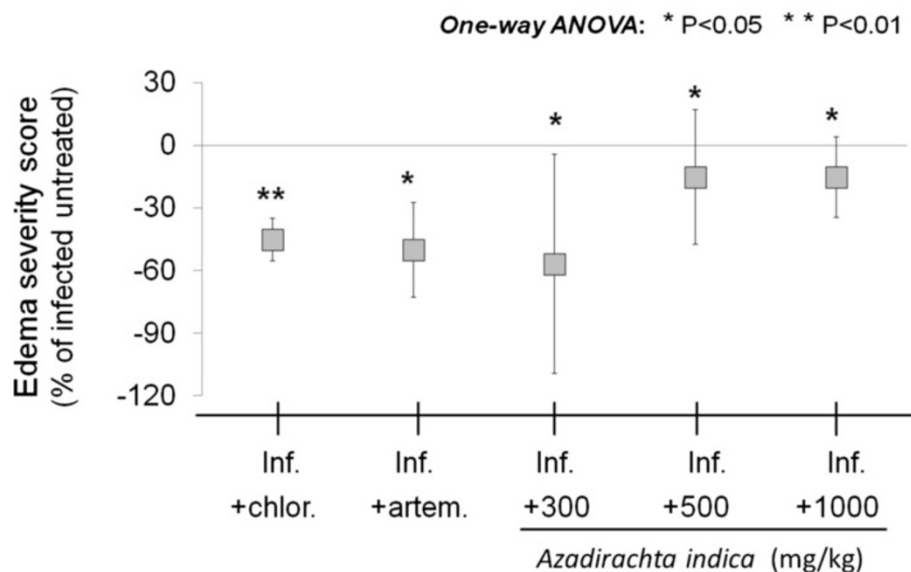


Figure 6 Brain oedema. A. Micrograph showing a representative case of highest brain oedema severity score. B. Effect of *Azadirachta indica* on the severity of brain oedema in *Plasmodium berghei*-infected (inf.) mice. Note the significant decrease in brain oedema severity following treatment with chloroquine (chlor.), artemether (artem.), or any of the doses of *A. indica* extract used. Data are mean \pm SEM.

Striking decrease in the expression of apoptosis markers

The markers of apoptosis studied were caspase 3, Fas, and Fas ligand, which are commonly used for the detection of apoptosis in neuronal populations [13,19]. *Azadirachta indica* extract induced a striking decrease in caspase 3, Fas, and Fas ligand expressions on pyramidal neurons of *P. berghei*-infected mice, particularly at the highest dose used. Considering that these markers are associated with the risk for neuron apoptosis [13,19], the present finding indicates that *A. indica* extract has anti-apoptotic effects on pyramidal neurons in experimental cerebral malaria. The latter effects probably account for at least part of the protective effects of the extract against pyramidal neuron death in treated *P. berghei*-infected mice.

Considering that the pro-inflammatory environment, and particularly infiltrating immune cells, contribute to the detrimental effects of cerebral malaria on neurons, including apoptosis in sensible neuronal populations [12,14,42], through extrinsic mediators of apoptosis such as TNF and NOS [12,43,44], we have addressed the effect of *A. indica* extract on these inflammatory triggers of apoptosis in *P. berghei*-infected mice.

Mitigation of neuroinflammatory triggers of apoptosis and brain oedema severity

Treatment with *A. indica* extract induced decreases in pyramidal neuron expression of TNF and NOS in *P. berghei*-infected mice as compared with disease control group,

suggesting that the extract modulated inflammation in the brain parenchyma. Besides, considering that brain oedema is a major causative of the inflammatory response in the brain parenchyma (through infiltrating danger or pathogen-associated molecules [10,11]), the decrease in oedema severity observed in groups treated with the extract in the present study, indicate that the aforementioned anti-inflammatory effects may be mediated, at least in part, by protective effects on brain vessels. These findings are in agreement with our precedent observations in Purkinje cells of *P. berghei*-infected mice, where *A. indica* also displayed neuroprotective effects [16]. Comparable effects of *A. indica* extract have been reported in other models of detrimental neuroinflammation associated with brain oedema, including cerebral post-ischemic reperfusion and hypoperfusion in rats [45,46]. Thus, the findings herein reported confirm that *A. indica* extract has neuroprotective effects in experimental cerebral malaria, and indicate that such effect may be mediated through protective effects on brain vessels and the consequent decrease in neuroinflammation severity. Not surprisingly and interestingly, chloroquine and artemether, drugs in use for cerebral malaria treatment in the field [37,47], also induced similar effects, corroborating their beneficial effects reported in humans, and pointing out at least part of the mechanisms accounting for these effects. It appears, therefore, that future studies aiming at unraveling the active principle(s) accounting for the beneficial effects of *A. indica* extract reported may provide key elements for the development of new drugs for cerebral malaria. Subjecting the bioactive agents of *Azadirachta indica* which have already been characterized, such as gedunin [33, 48], to a similar study may provide better insights into the neuroprotective effects herein described.

Conclusions

Azadirachta indica extract did not protect infected mice from death from cerebral malaria, although the highest doses slightly delayed death time. However, *A. indica* extract displayed neuroprotective effects mediated probably by mitigating oedema build up, and modulating both neuroinflammatory triggers of apoptosis and apoptosis signaling pathways. The use of extracts of *A. indica* in African and Asian folk medicine and practices for the treatment of malaria and cerebral malaria is justified. Characterization of the active principle(s) accounting for these effects will probably provide new drugs, which are particularly needed in the current context of increased chemoresistance to chloroquine.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MF, EAK, SAK, MAA, AM, PFSE, and YHA participated in the design of the experiment. SB and MF performed the experiments. MF, EAK, SAK, MAA, AM,

SB, YHA and PFSE participated in the preparation of the manuscript. MF and PFSE analysed the data, prepared the figures, and were in charge of the manuscript submission. All authors read and approved the final manuscript.

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References

1. Mishra SK, Newton CR: **Diagnosis and management of the neurological complications of falciparum malaria.** *Nat Rev Neurol* 2009, **5**:189–198.
2. Idro R, Marsh K, John CC, Newton CR: **Cerebral malaria: mechanisms of brain injury and strategies for improved neurocognitive outcome.** *Pediatr Res* 2010, **68**:267–274.
3. Idro R, Ndiritu M, Ogutu B, Mithwani S, Maitland K, Berkley J, Crawley J, Fegan G, Bauni E, Peshu N, Marsh K, Neville B, Newton C: **Burden, features, and outcome of neurological involvement in acute falciparum malaria in Kenyan children.** *JAMA* 2007, **297**:2232–2240.
4. Kihara M, Carter JA, Holding PA, Vargha-Khadem F, Scott RC, Idro R, Fegan GW, de Haan M, Neville BG, Newton CR: **Impaired everyday memory associated with encephalopathy of severe malaria: the role of seizures and hippocampal damage.** *Malar J* 2009, **8**:273.
5. Kariuki SM, Ikumi M, Ojal J, Sadarangani M, Idro R, Olotu A, Bejon P, Berkley JA, Marsh K, Newton CR: **Acute seizures attributable to falciparum malaria in an endemic area on the Kenyan coast.** *Brain* 2011, **134**:1519–1528.
6. Gwer S, Thuo N, Idro R, Ndiritu M, Boga M, Newton C, Kirkham F: **Changing trends in incidence and aetiology of childhood acute non-traumatic coma over a period of changing malaria transmission in rural coastal Kenya: a retrospective analysis.** *BMJ Open* 2012, **2**:e000475.
7. Kaiser K, Texier A, Ferrandiz J, Buguet A, Meiller A, Latour C, Peyron F, Cespuoglio R, Picot S: **Recombinant human erythropoietin prevents the death of mice during cerebral malaria.** *J Infect Dis* 2006, **193**:987–995.
8. Combes V, Coltel N, Faillle D, Wassmer SC, Grau GE: **Cerebral malaria: role of microparticles and platelets in alterations of the blood-brain barrier.** *Int J Parasitol* 2006, **36**:541–546.
9. Faillle D, El-Assaad F, Alessi MC, Fusai T, Combes V, Grau GE: **Platelet-endothelial cell interactions in cerebral malaria: the end of a cordial understanding.** *Thromb Haemost* 2009, **102**:1093–1102.
10. Pino P, Taoufiq Z, Nitchou J, Vouldoukis I, Mazier D: **Blood-brain barrier breakdown during cerebral malaria: suicide or murder?** *Thromb Haemost* 2005, **94**:336–340.
11. Bisser S, ON O-M-O-B, Toure FS, Taoufiq Z, Bouteille B, Buguet A, Mazier D: **Harboring in the brain: a focus on immune evasion mechanisms and their deleterious effects in malaria and human African trypanosomiasis.** *Int J Parasitol* 2006, **36**:529–540.
12. Lovegrove FE, Gharib SA, Patel SN, Hawkes CA, Kain KC, Liles WC: **Expression microarray analysis implicates apoptosis and interferon-**

- responsive mechanisms in susceptibility to experimental cerebral malaria. *Am J Pathol* 2007, **171**:1894–1903.
13. Lackner P, Burger C, Pfaller K, Heussler V, Helbok R, Morandell M, Broessner G, Tannich E, Schmutzhard E, Beer R: **Apoptosis in experimental cerebral malaria: spatial profile of cleaved caspase-3 and ultrastructural alterations in different disease stages.** *Neuropathol Appl Neurobiol* 2007, **33**:560–571.
 14. Toure FS, Ouwe-Missi-Oukem-Boyer O, Bisvigou U, Moussa O, Rogier C, Pino P, Mazier D, Bisser S: **Apoptosis: a potential triggering mechanism of neurological manifestation in *Plasmodium falciparum* malaria.** *Parasite Immunol* 2008, **30**:47–51.
 15. Teo JT, Swayne OB, Silber E: **Cerebellar ataxia after malaria.** *Neurology* 2009, **73**:73–74.
 16. Farahna M, Bedri S, Khalid S, Idris M, Pillai CR, Khalil EA: **Anti-plasmodial effects of *Azadirachta indica* in experimental cerebral malaria: Apoptosis of cerebellar Purkinje cells of mice as a marker.** *N Am J Med Sci* 2010, **2**:518–525.
 17. Duque V, Seixas D, Ventura C, Da CS, Melico-Silvestre A: ***Plasmodium falciparum* malaria, bilateral sixth cranial nerve palsy and delayed cerebellar ataxia.** *J Infect Dev Ctries* 2012, **6**:290–294.
 18. Potter S, Chan-Ling T, Ball HJ, Mansour H, Mitchell A, Maluish L, Hunt NH: **Perforin mediated apoptosis of cerebral microvascular endothelial cells during experimental cerebral malaria.** *Int J Parasitol* 2006, **36**:485–496.
 19. Potter SM, Chan-Ling T, Rosinova E, Ball HJ, Mitchell AJ, Hunt NH: **A role for Fas-Fas ligand interactions during the late-stage neuropathological processes of experimental cerebral malaria.** *J Neuroimmunol* 2006, **173**:96–107.
 20. Wiese L, Kurtzhals JA, Penkowa M: **Neuronal apoptosis, metallothionein expression and proinflammatory responses during cerebral malaria in mice.** *Exp Neurol* 2006, **200**:216–226.
 21. Schmutzhard J, Kositz CH, Glueckert R, Schmutzhard E, Schrott-Fischer A, Lackner P: **Apoptosis of the fibrocytes type 1 in the spiral ligament and blood labyrinth barrier disturbance cause hearing impairment in murine cerebral malaria.** *Malar J* 2012, **11**:30.
 22. Mackintosh CL, Beeson JG, Marsh K: **Clinical features and pathogenesis of severe malaria.** *Trends Parasitol* 2004, **20**:597–603.
 23. Medana IM, Idro R, Newton CR: **Axonal and astrocyte injury markers in the cerebrospinal fluid of Kenyan children with severe malaria.** *J Neurol Sci* 2007, **258**:93–98.
 24. Newton PN, Green MD, Mildenhall DC, Plancon A, Nettey H, Nyadong L, Hostetler DM, Swamidoss I, Harris GA, Powell K, Timmermans AE, Amin AA, Opuni SK, Barbereau S, Faurant C, Soong RC, Faure K, Thevanayagam J, Fernandes P, Kaur H, Angus B, Stepniewska K, Guerin PJ, Fernandez FM: **Poor quality vital anti-malarials in Africa - an urgent neglected public health priority.** *Malar J* 2011, **10**:352.
 25. Cheeseman IH, Miller BA, Nair S, Nkhoma S, Tan A, Tan JC, Al SS, Phyo AP, Moo CL, Lwin KM, McGready R, Ashley E, Imwong M, Stepniewska K, Yi P, Dondorp AM, Mayxay M, Newton PN, White NJ, Nosten F, Ferdig MT, Anderson TJ: **A major genome region underlying artemisinin resistance in malaria.** *Science* 2012, **336**:79–82.
 26. Nayyar GM, Breman JG, Newton PN, Herrington J: **Poor-quality antimalarial drugs in southeast Asia and sub-Saharan Africa.** *Lancet Infect Dis* 2012, **12**:488–496.
 27. Ekanem OJ: **Has *Azadirachta indica* (Dogonyaro) any anti-malarial activity?** *Niger Med J* 1978, **8**:8–11.
 28. El Tahir A, Satti GM, Khalid SA: **Antiplasmodial activity of selected Sudanese medicinal plants with emphasis on *Maytenus senegalensis* (Lam.) Exell.** *J Ethnopharmacol* 1999, **64**:227–233.
 29. Hashmat I, Azad H, Ahmed A: **Neem (*Azadirachta indica* A. Juss) - A nature's drugstore: an overview.** *Int Res J Biol Sci* 2012, **1**:76–79.
 30. Dike IP, Obembe OO, Adebiji FE: **Ethnobotanical survey for potential anti-malarial plants in south-western Nigeria.** *J Ethnopharmacol* 2012, **144**:618–626.
 31. Belayneh A, Asfaw Z, Demissew S, Bussa NF: **Medicinal plants potential and use by pastoral and agro-pastoral communities in Erer Valley of Babile Wereda, Eastern Ethiopia.** *J Ethnobiol Ethnomed* 2012, **8**:42.
 32. Obaseki O, Fadunsin J: **The antimalarial activity of *Azadirachta indica*.** *Fitoterapia* 1986, **57**:247–251.
 33. Khalid SA, Duddeck H, Gonzalez-Sierra M: **Isolation and characterization of an antimalarial agent of the neem tree *Azadirachta indica*.** *J Nat Prod* 1989, **52**:922–926.
 34. Isah AB, Ibrahim YK, Iwalewa EO: **Evaluation of the antimalarial properties and standardization of tablets of *Azadirachta indica* (Meliaceae) in mice.** *Phytother Res* 2003, **17**:807–810.
 35. Udeinya JI, Shu EN, Quakyi I, Ajayi FO: **An antimalarial neem leaf extract has both schizonticidal and gametocytocidal activities.** *Am J Ther* 2008, **15**:108–110.
 36. Lucantoni L, Yerbanga RS, Lupidi G, Pasqualini L, Esposito F, Habluetzel A: **Transmission blocking activity of a standardized neem (*Azadirachta indica*) seed extract on the rodent malaria parasite *Plasmodium berghei* in its vector *Anopheles stephensi*.** *Malar J* 2010, **9**:66.
 37. de Miranda AS, Brant F, Machado FS, Rachid MA, Teixeira AL: **Improving cognitive outcome in cerebral malaria: insights from clinical and experimental research.** *Cent Nerv Syst Agents Med Chem* 2011, **11**:285–295.
 38. Highley JR, Walker MA, McDonald B, Crow TJ, Esiri MM: **Size of hippocampal pyramidal neurons in schizophrenia.** *Br J Psychiatry* 2003, **183**:414–417.
 39. Basir R, Rahiman SF, Hasballah K, Chong W, Talib H, Yam M, Jabbarzare M, Tie T, Othman F, Moklas M, Abdullah W, Ahmad Z: ***Plasmodium berghei* ANKA infection in ICR mice as a model of cerebral malaria.** *Iran J Parasitol* 2012, **7**:62–74.
 40. Lekic T, Rolland W, Manaenko A, Krafft PR, Kamper JE, Suzuki H, Hartman RE, Tang J, Zhang JH: **Evaluation of the hematoma consequences, neurobehavioral profiles, and histopathology in a rat model of pontine hemorrhage.** *J Neurosurg* 2013, **118**:465–477.
 41. Spriet A, Beiler D: **When can 'non significantly different' treatments be considered as 'equivalent'?** *Br J Clin Pharmacol* 1979, **7**:623–624.
 42. Hunt NH, Golenser J, Chan-Ling T, Parekh S, Rae C, Potter S, Medana IM, Miu J, Ball HJ: **Immunopathogenesis of cerebral malaria.** *Int J Parasitol* 2006, **36**:569–582.
 43. Alvarez S, Blanco A, Fresno M, Munoz-Fernandez MA: **TNF-alpha contributes to caspase-3 independent apoptosis in neuroblastoma cells: role of NFAT.** *PLoS One* 2011, **6**:e16100.
 44. Bienvenu AL, Gonzalez-Rey E, Picot S: **Apoptosis induced by parasitic diseases.** *Parasit Vectors* 2010, **3**:106.
 45. Yanpallewar S, Rai S, Kumar M, Chauhan S, Acharya SB: **Neuroprotective effect of *Azadirachta indica* on cerebral post-ischemic reperfusion and hypoperfusion in rats.** *Life Sci* 2005, **76**:1325–1338.
 46. Vaibhav K, Shrivastava P, Khan A, Javed H, Tabassum R, Ahmed ME, Khan MB, Moshahid KM, Islam F, Ahmad S, Siddiqui MS, Saffhi MM, Islam F: ***Azadirachta indica* mitigates behavioral impairments, oxidative damage, histological alterations and apoptosis in focal cerebral ischemia-reperfusion model of rats.** *Neurol Sci* 2013, **34**(8):1321–1330.
 47. Ibezim EC, Odo U: **Current trends in malarial chemotherapy.** *Afr J Biotechnol* 2008, **7**:349–356.
 48. Khalid SA, Farouk A, Geary TG, Jensen JB: **Potential antimalarial candidates from African plants: and in vitro approach using *Plasmodium falciparum*.** *J Ethnopharmacol* 1986, **15**:201–209.

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