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Oral administration of Coenzyme Q_{10} protects mice against oxidative stress and neuro-inflammation during experimental cerebral malaria

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ABSTRACT

In animal model of experimental cerebral malaria (ECM), the genesis of neuropathology is associated with oxidative stress and inflammatory mediators. There is limited progress in the development of new approaches to the treatment of cerebral malaria. Here, we tested whether oral supplementation of Coenzyme Q_{10} (CoQ_{10}) would offer protection against oxidative stress and brain associated inflammation following *Plasmodium berghei* ANKA (PbA) infection in C57BL/6 J mouse model. For this purpose, one group of C57BL/6 mice was used as control; second group of mice were orally supplemented with 200 mg/kg CoQ_{10} and then infected with PbA and the third group was PbA infected alone. Clinical, biochemical, immunoblot and immunological features of ECM was monitored. We observed that oral administration of CoQ_{10} for 1 month and after PbA infection was able to improve survival, significantly reduced oedema, TNF-α and MIP-1β gene expression in brain samples in PbA infected mice. The result also shows the ability of CoQ_{10} to reduce cholesterol and triglycerides lipids, levels of matrix metalloproteinases-9, angiopoietin-2 and angiopoietin-1 in the brain. In addition, CoQ_{10} was very effective in decreasing NF-κB phosphorylation. Furthermore, CoQ_{10} supplementation abrogated Malondialdehyde, and 8-OHDG and restored cellular glutathione. These results constitute the first demonstration that oral supplementation of CoQ_{10} can protect mice against PbA induced oxidative stress and neuro-inflammation usually observed in ECM. Thus, the need to study CoQ_{10} as a candidate of antioxidant and immunomodulatory molecule in ECM and testing it in clinical studies either alone or in combination with antimalaria regimens to provide insight into a potential translatable therapy.

1. Introduction

1.1. Background information

Malaria is a global health disaster that has continued claiming many lives despite control measures being in place. In 2016 alone, approximately 216 million new cases of malaria were reported with death rates hitting ever high at 445, 000 since 2015 [1]. *P. falciparum* is one of the five human species that cause cerebral malaria (CM) which is a life threatening complication that often affects infants and travelers from non-endemic areas.

Cerebral malaria is characterized by delirium, body ache, fever, coma and ultimately impaired consciousness in patients thus a complex neurological syndrome whose pathology is mediated by inflammatory processes following infection by the parasites [2]. Hypothesis which has been advanced to date clearly shows that pathogenesis and alteration in tissue pathophysiology of CM is due to sequestration of the parasite in microvasculature, especially the adherence of parasitized erythrocytes in the endothelial lining of the brain [3]. Additionally, infiltrated leukocytes, pro-inflammatory cytokines and other inflammatory mediators and other mediators also play a major role in the pathogenesis of CM. Although the mechanism that mediate the pathogenesis of CM has been intensively investigated, many grey areas still exist which have not been unlocked in order to conclusively define the exact role of cellular

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and molecular pathogenesis in CM [2]. On the other hand it has been argued out that this phenomenon might be due to differences in the activation status of immune cells, host genetics, intricacy of host-pathogen interplay and uniqueness of malaria parasite factors [4]. This intricacy has been affirmed by both mouse model and clinical studies [5–7]. Furthermore, oxidative stress markers have been observed to be significantly increased in infected humans and the brains of PbA-infected mice [8–10]. Additionally, an increased level of oxidative stress has also been observed in hippocampus region of the brains in PbA infected mice with concomitant augmentation in the levels of protein carbonyl that contributes to oxidative damage of proteins [11,12].

It is noteworthy that Reactive oxygen species (ROS) like lipid peroxides, hydroxyl radicals, hydrogen radicals and superoxide ions are known to mediate oxidative stress that cause assault in a number of organs and host tissues. Such ROS are generated when immune cells like macrophages, monocytes and neutrophils are activated and recruited during plasmodium infection [13]. Moreover, compelling evidence has shown that hemoglobin breakdown by the parasite and release of free heme during plasmodium infection is the genesis of heightened production of ROS that ultimately contribute to brain cells damage during cerebral malaria [4, 13].

With this assumption in mind, at structural level, it is expected that there will be inflammation, mitochondrial and proteasomal dysfunction in periods of harsh oxidative stress that culminates to the events that results in brain injury [14,15]. Similarly, significant increase in malondialdehyde a marker of oxidative stress together with decrease in levels of GSH and catalase has been observed in cerebral malaria [16]. More importantly, it has been possible to study *in vivo* the presence of oxidative stress during experimental cerebral malaria by use of transgenic Keap1-dependent Oxidative stress Detector, No-48-luciferase (OKD-48) mice [17]. Whereas constitutive antioxidant system plays a vital role in scavenging free radicals, generation of excessive oxidative stress will impair the defense system with putative resultant neurological dysfunction generally observed in CM. Transient supplementation with anti-oxidants reinforces these systems and protects mice against ECM [18]. Likewise, treatment of oxidative stress induction during plasmodium infection with pro-oxidants protects against malaria infection [19].

Progress in the treatment of CM has not been impressive to say the least. Treatment of CM relies heavily on artemisinin combination therapy (ACT), nonetheless, the situation is further compounded by the fact that resistance to ACTs have been reported in South East Asia and sub-Saharan Africa. Needless to say, the need for safer adjunct therapy including anti-oxidative drugs is of vital importance. It is therefore paramount that additional research and development efforts be geared towards the search of novel drugs and enhancement of current drugs by making them more effective.

Coenzyme Q_{10} is a cofactor that transfers electrons in the mitochondria from complex one to complex III and effectively promotes mitochondrial activity while mitigating oxidative stress. In addition to its beneficial effect on mitochondrial protection, CoQ_{10} is a powerful antioxidant that has the capability to cross the blood brain barrier. There is an increasing interest in the potential usefulness of co-enzyme Q_{10} to treat neurodegenerative diseases such as Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis [20]. Specifically Coenzyme Q_{10} supplementation demonstrates a robust effect in mitigating neuro-inflammation in neurodegenerative diseases and reducing toxicity associated with a number of drugs and immune cells [21,22]. Coenzyme Q_{10} has been identified as a seemingly unifying ameliorative factor in pathological phenomenon in Human African Trypanosomiasis, hence positioning Coenzyme Q_{10} as a promising antioxidant therapeutic [23,24]. The reduced form of CoQ_{10} ($Q_{10}H_2$) serves as a potent antioxidant of lipid membranes and has been identified as a modulator of inflammatory gene expression *in vitro* [25]. However, the exact role of CoQ_{10} in brain associated inflammation and oxidative stress following *Plasmodium* infection currently has not been determined. The antioxidant system therefore potentially presents critical pathways that can be targeted in development of tools for management of neurological manifestations due to CM or related drugs that deserve further scrutiny. Here, we demonstrate that oral administration of 200 mg/kg CoQ_{10} can protect mice infected with PbA against oxidative stress and neuro-inflammation usually observed in ECM. Since oxidative stress can exacerbate the pathophysiology usually witnessed during CM, Coenzyme Q_{10} delivery may constitute a relevant strategy in cerebral malaria treatment.

2. Materials and methods

2.1. Ethics statement and mice

This study utilized C57BL/6 J mice which seem to reflect some aspects of human cerebral malaria disease. The experimental work and design for this study was based on the rules and guidelines of animal husbandry [26]. Three-four weeks-old female C57BL/6 J mice were purchased from International livestock research institute (ILRI). Approval of all experimental procedures and protocols involving mice were obtained from local regulatory agencies. The mice were kept under specific pathogen free environment, wood-chippings were provided as bedding material, and were maintained on mice pellets and water *ad libitum* at room temperature. All experimental procedures and protocols involving mice were also reviewed and approved by Institutional review Committee (IRC) of Institute of Primate Research, (ISERC/08/2017), Karen, Nairobi Kenya.

2.2. Experimental design

The mice were allocated randomly into cages with $n = 5–6$ mice per group while survival experiment was conducted with 8 mice. Mice were divided into three groups: Group one wild type (WT) naïve control, experimental group two: Wild type + PbA infection and experimental group three: Wild type + Coenzyme Q_{10} + PbA. Infection, treatment and assessment of the health status were performed sequentially.

2.3. Euthanization of mice

Euthanization was carried out by injecting mice with ketamine (50 mg/mL) and Rompun in a ratio of 4:1 intramuscularly, after which mice were opened and then perfused with PBS. Humane endpoints were monitored to limit suffering. A sick animal with clear health problems like unarousable coma, retinal haemorrhages, dysconjugate gaze, pouting, decerebrate rigidity, respiratory distress and convulsions prior to treatment and infection were euthanized by cervical dislocation under isofluran.

2.4. Treatment of mice with 200 mg/kg CoQ_{10}

Oral administration of 200 mg/kg of Coenzyme Q_{10} was done daily for 1 month prior to infection with PbA to the experimental group three of mice, using a gavage needle and continued thereafter until day 6 of ECM. The CoQ_{10} solutions were prepared immediately before use and were protected from the light before administration to the animals. Briefly, Coenzyme Q_{10} solution (Zambom Group S.p.A., Italy) was prepared by directly dissolving it in olive oil.

2.5. Plasmodium berghei ANKA (PbA) infection studies

All experiments in this study made use of *Plasmodium berghei* ANKA [27]. All experimental mice in group two and three were intravenously (i.v) infected with $5 \times 10^{\wedge 4}$ pRBC that was obtained from donor mice (stock mice) infected with stock solution of PbA, which was stored in liquid nitrogen as pRBC in solution containing 10% glycerol. Parasitaemia in infected mice was monitored daily with 5% Giemsa stained

blood smears.

2.6. Brain oedema assessment

The brain water content was analyzed to determine cerebral oedema and was estimated by employing the wet-dry method. On day 6 of ECM, mice were anaesthetized and brain tissues extracted. The brains were immediately weighed to measure the wet weight. The brains were then incubated at 80 °C overnight; the brains were then weighed to obtain the dry weight. The water content was calculated using the following formula: % of water content = [(wet weight − dry weight)/wet weight] × 100 [28].

2.7. Haematocrit quantification

For determination of hematocrits, samples of blood 6 days post infection (dpi) were collected from each mouse by tail snip into 100 μL heparinized capillary tubes or PCV determination as per the method of [29]. After blood collection, the capillary tubes were sealed with plasticin at one end and centrifuged in a haematocrit centrifuge (Hawksley H England) at 10,000 rpm (RPM) for 5 min. PCV was then read using a micro-haematocrit reader and expressed as a percentage (%) of the total blood volume.

2.8. Sample preparation

Snap-frozen whole brains were homogenized on ice water (4 °C) in a mixture of 0.5 mLs of 0.25 M sucrose, 5 mM Hepes-Tris, pH 7.4, with protease inhibitor cocktail to a final concentration of 10% (*w/v*). The homogenates were aliquoted into 0.5 microfuge tubes (to avoid excessive freeze-thaw process) and stored in liquid nitrogen for analysis.

2.9. Assessment of 8-hydroxy-2-deoxyguanosine (8-OHdG)

Perfused brain samples stored in liquid nitrogen were homogenized in clean tubes containing 1.5 mL 0.25 M sucrose. The brain homogenate was centrifuged at 600 × g for 10 min (to remove cells, debris and nuclei). The resulting supernatant was transferred into clean sterile tubes and then kept on ice. The remaining pellets were re-suspended with 1.5 mL of 0.25 m sucrose and centrifuged at the same speed as before. The resulting supernatant was combined with the first supernatant and then centrifuged at 15,000 × g for 5 min. The resulting supernatant was discarded and the packed pellets were re-suspended in 1.0 mL of 1 × cytosol extraction buffer, followed by incubation on ice for 10 min and centrifugation at 700 × g for 10 min at 4 °C (This step was to remove nuclei and other intact cells in pellet). The resulting supernatant was transferred to clean sterile tubes, and centrifuged at 10,000 × g for 30 min at 4 °C. The supernatant was discarded followed by re-suspending the pellets with 1 mL of extraction buffer (1 × cytosol) and then centrifuged at 10,000 × g for 30 min at 4 °C again. The supernatant was removed and the remaining pellet represented isolated mitochondria. The mitochondria was lysed in 30 μL of the mitochondrial lysis buffer then 15 μL enzyme B (Enzyme B mix degrade all proteins and DNases) was added and incubated at 50 degree C overnight. Absolute ethanol (100 μL) was added, mixed and then incubated at −20 °C for 10 min. The resulting solution was centrifuged in microcentrifuge at 10000 × g for 5 min at room temperature. The supernatant was removed and the pellet was washed 2 times with 1 mL of 70% ethanol. The trace amount of ethanol was removed using pipette tip. The tube was air dried for 5 min. The mtDNA was re-suspended in 20 μL TE buffer. The mtDNA and nDNA was then purged with a nitrogen steam, followed by incubating the mixture at 37 °C for 1 h in order to digest the DNA to nucleotides. The resulting mixture samples were re-suspended with 500 mmol/l Tris-HCl (pH 8.0), 10 mmoL/L MgCl2, and 0.6 units of alkaline phosphatase followed by further incubation at 37 °C for 1 h to hydrolyze the nucleotides to nucleosides. The nucleoside samples for both mtDNA and nDNA were used for the analysis of 8-OHdG levels from brain by competitive Enzyme linked Immunosorbent Assay (ELISA) kit (8-OHdG Check; Japan Institute for the Control of Aging, Fukuroi).

2.10. Analysis of free oxyhaemoglobin

On day six of ECM mice were sacrificed and the blood was obtained intracardially and transferred into EDTA coated tubes and centrifuged at 5000 rpm, 15 min at RT. The free oxyhemoglobin levels in plasma were determined as per the method of [30] with slide modification. Briefly, about 500 μL of plasma was transferred to a quartz microcuvette, and the absorbance was adjusted with the water blank. The absorbance of free oxyhemoglobin was then obtained at a wavelength on the spectrophotometer at 578 nm.

2.11. Glutathione (GSH) assay

Total, reduced and oxidized GSH content was determined by employing the method of [31] with slight modification. Briefly, the brain homogenates were mixed with a solution containing sulphosalicylic acid (4.31% (*w/v*)) and 0.25 mM EDTA. The GSH in the homogenates was determined chemically by reacting the GSH therein with Ellman's reagent (DTNB) and measuring the absorbance of the reaction product at 412 nm using a multi-detection microplate reader (Bio-Tek Synergy HT).

2.12. Determination of endothelial barrier integrity

Plasma concentration of endothelial integrity biomarkers (MMP-9 and MMP-1) was measured by sandwich ELISA. At day 6 of ECM, mice were sacrificed and blood was obtained intracardially. Blood samples were centrifuged at 800 x g for ten minutes and plasma aliquots were transferred to clean tubes. Plasma levels of MMP-9 and MMP-1 were quantified by spectrometer (Spectra Max 340pc384, Molecular Devices, Sunnyvale, USA).

2.13. Western blot for determination of phosphorylation of signal proteins

Brains were homogenized on ice water (4 °C) in 0.5 mL of 0.25 M sucrose, 5 mM Hepes-Tris, pH 7.4, with protease inhibitor cocktail to a final concentration of 10%. The homogenates were aliquoted into 0.5 microfuge tubes (to avoid excessive freeze-thaw process) and stored in liquid nitrogen until required for analysis. Once the protein concentrations in the brains was determined as described above, aliquots of those fractions were subjected to western blot analyses for IKKα/β and ph NF-κB phosphorylation signal proteins. Proteins were separated on pre-cast gradient gels (4–15% Tris-HCl, SDS Polyacrylamide) at 125 constant volts using the BioRad mini-gel systems. After electrophoresis, the proteins were transferred and bound to a nitrocellulose membrane (Millipore) at 25 V for 150 min. Once the transfer was complete, the membranes were incubated in 3% (*w/v*) fat-free milk (blocking solution) for four hours at room temperature. The blocking solution was discarded and the blots were incubated in solutions containing, primary antibodies for Phospho-NF-κB Antibody and Phospho-IKKα/β Antibody II (Cell Signaling Technology, USA), followed by their respective peroxidase-conjugated secondary antibodies. The protein-antibody complexes were visualized with 3.3, diaminobenzidine (DAB) reagents using the peroxidase substrate kit. The relative levels of these proteins were determined using a pixel density analysis software (NIH, UN-SCAN-IT). Anti-Human Tubulin beta (US Biological, USA, Swampscott, Massachusetts) was used to confirm equal sample loading for the brain homogenates.

2.14. Liver function assay and lipid profile determination

At day 6 post infection, blood was collected into anti-coagulant free sterile tubes from different experimental groups of mice. The blood was left at room temperature until it coagulated and then centrifuged at 8000 x g for 10 min. Serum that was obtained was transferred into new eppendorf tube and immediately followed by measurement of liver enzymes, serum glutamic oxaloacetate transaminase (sGOT) and serum glutamic pyruric transaminase (sGPT) and alkaline phosphatase (ALP). Levels of total Cholesterol, triglycerides, low density lipoprotein and high density lipoprotein were also analyzed with the Reflotron test system (Roche).

2.15. RNA extraction and real-time PCR

Total RNA was obtained from frozen brain samples by the Trizol (Invitrogen, Carlsbad, CA, USA) method according to the manufacturer's instructions. DNase I was added to the extracted RNA in order to digest DNA that could be present. The resulting total RNA was quantified by measuring at absorbance of 260/280 nm using spectrometer (Spectra Max 340pc384, Molecular Devices, Sunnyvale, USA) and was also reverse transcribed with the oligo(dT) primer into complementary DNA (cDNA). In brief, approximately one-fifth volume that constitutes the reverse transcription reaction mixture was used for real-time PCR with the primer pairs for **hemoxygenase-1 (HO-1)** (FW: ggtcctcactct-cagcttcct RV: ccaggcaagattctcccttac) i**NOS** (FW: cagctgggctgtacaaaccctt RV: tcacagaactgaacgtgatgc) **TNF-α** (FW: catcttctcaaaattcgagtgacaa RV: tgggagtagacaaggtacaaccc) **IL-1β** FW: caaccaacaagtgatattctccatg RV: gatccacactctccagctgca. The cDNA was amplified in a Rotor- Gene 3000 or 6000 (Corbett Research, Hilden, Germany) using SYBR Green (Invitrogen, Eugene, Oregon, USA) and gene-specific primers.

Calculation of fold change according to [32].

$$\text{Fold change (normalized)} = \frac{(\mathbf{E}p - target)^{\Delta CT-target}}{(\mathbf{E}p\ housekeeping)^{\Delta CT-housekeeping}}$$

$C_{T\text{-target}}$ = ($C_{T\ Naive}$ -$C_{T\text{-PbAMA infected}}$)CT-housekeeping = (C T naive housekeeping -CT-housekeeping PbAMA infected)

2.16. Quantification of nitric oxide in brain tissue using Griess assay

The brains were harvested and homogenized in a PBS-buffer containing a protease-inhibitor cocktail. Nitric oxide (NO) was quantified as nitrite. Nitrates were reduced to nitrites by enzymatic conversion by nitrate reductase (SIGMA). The level of nitrites was determined by Griess method. Briefly Griess reagent was prepared by mixing equal volumes of components A [N-(1-naphthyl) ethylenedi-amine] and B (sulfanilic acid). 10 µL of freshly prepared Griess reagent was mixed with 75 µL of sample/Aqua dest. (Blank)/standard (serial dilution 10–1 µM) and 65 µL of Aqua dest., to have a total volume of 150 µL. The mix was incubated for 30 min in the dark. NO production was then quantified by the colour change at 548 nm using a spectrometer (Spectra Max 340pc384, Molecular Devices, Sunnyvale, USA).

2.17. Assessment of malondiadehyde and diene levels

To determine lipid peroxidation levels in murine brains during ECM, malondialdehyde levels were measured by assays of thiobarbituric acid reactive species TBARS [33] and the formation of dieneconjugated species [34]. Brains from mice at day 6 of ECM were homogenized in cold phosphate buffer, pH 7.4 with BHT (final concentration 0.2%). Briefly, the brain homogenate samples (0.5 mL) were mixed with equal volume of thiobarbituric acid 0.67% (Sigma Chemical, St. Louis, MO) and then heated at 92-96 °C for 30 min. Thiobarbituric acid reactive species production was then quantified at 535 nm using a spectrometer (Spectra Max 340pc384, Molecular Devices, Sunnyvale, USA). To evaluate the formation of dieneconjugate, lipids were extracted by panel on chloroform: methanol (2:1, v:v) the resulting organic phase was quantified at 234 nm. Results were expressed as malondialdehyde and diene equivalents per milligram of protein (BCA assay).

2.18. Statistical analysis

Data was analyzed using Graph pad prism 5.0 software package. The survival rate was analyzed with Log-rank (Mantel-Cox) Test. Significance of differences between the means for treated, infected and control mice was determined by One way analysis of variance and Bonferroni post-test was done to check the differences among the group means. Results was given as mean + SEM with significance level set at $p < .05$.

3. Results

3.1. Oral supplementation of Co-Q_{10} improves the survival rate in C57BL/6 mice infected with Plasmodium berghei ANKA

To test the hypothesis that oral administration of 200 mg/kg of CoQ_{10} might have a beneficial effect on the outcome of experimental cerebral malaria, C57BL/6 J mice were orally supplemented with 200 mg/kg/day of CoQ_{10} for 30 days (and then continued after infection) these mice were inoculated with 5×10^4 PbA-infected RBCs together with wild type mice that never received Co-Q_{10}. All treated and untreated mice developed blood-stage infection. All CoQ_{10} un-supplemented mice infected with PbA developed severe neurological symptoms associated with ECM from 5 to 6 days p.i, and almost invariably died rapidly thereafter (in a time phase of 6–9 days). As shown in Fig. 1, in WT-PbA infected mice, only 4/8 (50%) mice survived past day 7 and 0/8 (0%) survived past day 8). In contrast, mice orally administered with CoQ_{10} by oral gavage were potently resistant to the development of ECM, with 6/8 (75%) surviving past day 7 and 5/8 (62.5%) surviving past day 9 (Fig. 1).Therefore, these results, remarkably indicate that despite the fact that antioxidant treatment has limited clinical relevance; oral administration of Co-Q_{10} prevented lethal ECM in majority of mice infected with PbA.

One group of C57BL/6 J (WT) mice were orally administered with 200 mg/kg of CoQ_{10} for 30 days and then continued after infection with 5×10^4 iRBCs i.v together with WT mice that did not receive CoQ_{10}. They were then monitored from d + 5 p.i for survival. The CM phase (d6–9) is indicated by the hatched bar. The survival rate was analyzed with Log-rank (Mantel-Cox) Test ($n = 8$).

3.2. Reduction in brain oedema in PbA-infected Coenzyme Q_{10} orally administered mice

A landmark deleterious event in PbA infection is the occurrence of brain oedema [35]. Since cerebral oedema are some of the neurological features observed during severe malaria and has a direct linkage to up-

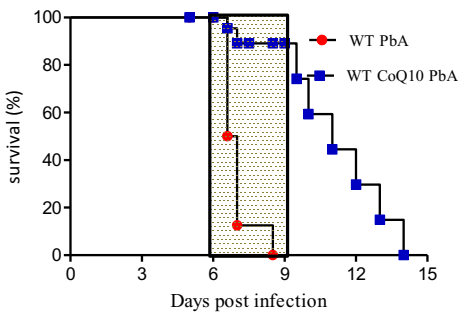

Fig. 1. Oral administration of CoQ_{10} increases the survival rate during murine PbA infection.

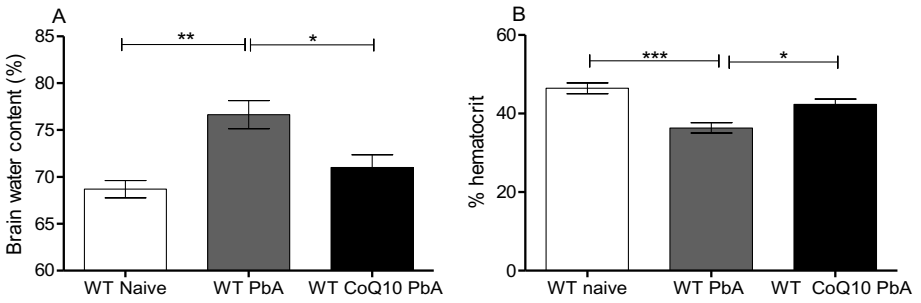

Fig. 2. Oral administration of CoQ_{10} decreases oedema in the brains of PbA-infected mice. C57BL/6J mice were orally administered with CoQ10 or none and then inoculated with 5×10^4 red blood cells infected with PbA, and euthanized on day 6 p.i. The brain oedema (A) and haematocrit (B) were evaluated. Brains were extracted, and oedema was analyzed based on the measurement of brain water content estimated by the wet-dry method. Blood was drawn from the tail and then centrifuged to obtain the PCV values. Data are presented as mean of each group ± SEM and are representative of at least two independent experiments. Statistics = One-way Anova and Bonferroni for post test. Asterisks indicates significant differences between the groups indicated by brackets (*$p \leq .05$). n = 5–6 mice per group. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

regulation of capillary permeability that leads to intravascular fluid loss [36], we sought to determine whether CoQ_{10}-enhanced survival from ECM was associated with reduced edema in the brains. Brain oedema was analyzed based on the measurement of brain water content estimated by the wet-dry method. It was observed that Wild type PbA infected mice presented statistically higher brain water content compared with CoQ_{10} orally administered PbA infected mice (Fig. 2A). These results suggest that administration CoQ_{10} was able to reduce but not completely prevent oedema generation into the brain tissue upon PbA-infection. The group of mice receiving Coenzyme Q_{10} presented haematocrit levels similar to uninfected control animals (Fig. 2B). This result demonstrates the effect of protecting against RBC destruction can be linked to the antioxidant effect of CoQ_{10}.

3.3. Inflammatory transcripts were abrogated in the brains of CoQ_{10} supplemented PbA infected mice

The expressions of transcripts encoding inflammatory cytokines that are associated with ECM were investigated in this study. Brain samples were harvested from both controls, CoQ_{10} supplemented and un-supplemented PbA infected mice into Trizol solution and stored at -80 °C. RNA was extracted and then converted to cDNA. Cytokine-specific primers and real time PCR were used to quantify relative abundance of cytokine transcripts in the brains. In agreement with pronounced aggravation of ECM in un-supplemented CoQ_{10} PbA infected group, transcripts encoding TNF-α mRNA were significantly increased in the PbA infected mice in comparison with CoQ_{10} supplemented PbA infected group 6 d p.i. (Fig. 3A). However, there was no significant difference in the abundance of transcripts encoding and IL-1β and iNOS mRNA in the brains of PbA infected mice in comparison with CoQ_{10} supplemented PbA infected mice (Fig. 3B-C).

3.4. Significant decrease in MPP-9 in mice administered with CoQ_{10} on day 6 of ECM as compared with PbA infected mice

Growing evidence has shown that matrix metalloproteinases (MMPs) play a crucial role in malaria in both animal and human disease models [37,38]. Notably, high levels of MMP-9 and the tissue inhibitors of metalloproteinases (TIMP-1) have been found in serum of patients with severe malaria and are related to disease severity [39]. Therefore, we compared the levels of MMP-9 and TIMP-1 in plasma between CoQ_{10} supplemented and un-supplemented PbA infected at 6 days p.i. In the brains of C57Bl/6 mice supplemented with CoQ_{10} infected with PbA, the levels of MMP-9 were significantly attenuated when compared to mice without CoQ_{10} but infected with PbA (Fig. 4A). In contrast, the levels of TIMP-1 were comparable across the two groups (Fig. 4B).

3.5. Endothelial biomarkers differ between CoQ_{10} treated and untreated PbA infected mice

Since high levels of angiogenic factors: angiopoietin-2 (Ang-2) and angiopoietin-1 (Ang-1) are associated with malaria disease severity [40]. An attempt to determine the putative impact of CoQ_{10} on these angiogenic factors was made. To address this question, the levels of these angiogenic factors in mice infected with PbA were determined and compared to those mice supplemented with CoQ_{10} and then infected with PbA at 6 days P.i. The levels of Ang-1 were significantly reduced in CoQ_{10} treated mice in comparison with un-treated PbA infected mice (Fig. 5A). In contrast, there was no statistical significance difference that was observed in respect to the levels of Ang-2 between treated and untreated groups (Fig. 5B). On the other hand, there was a marked significant decrease in the ratio of the angiogenic factors in CoQ_{10} treated group in comparison to untreated group (Fig. 5C). Also levels of Vcam-1 were assessed. There were no significant differences in

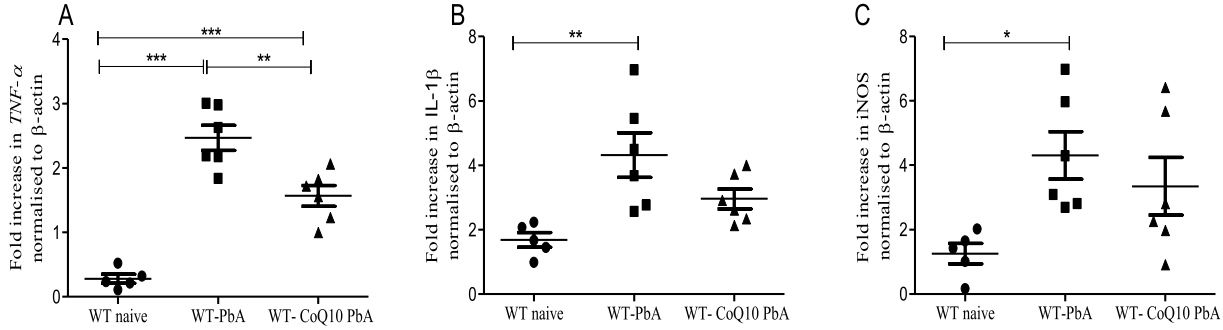

Fig. 3. CoQ_{10} administration alters the inflammatory transcript. C57BL/6J mice were orally supplemented with 200 mg/kg/day of CoQ_{10} for 30 days and simultaneously infected as WT mice with 5×10^4 PbA iRBC. After 6 days of infection, RNA from the brain were analyzed and the level of immune response determined. (A) Expression levels of TNF-α; (B) IL-1β and (C) iNOS in the brain were determined by qPCR. The relative gene expression levels were calculated by reference to the β-actin in each brain sample, using the threshold cycle (Ct) method and β-actin levels were comparable in all brain samples. Bars shows mean of each group ± SEM and are representative of at least two independent experiments. Expression level of markers of inflammatory immune response was compared by ANOVA, followed by a Bonferroni post-test (indicated level of significance: *$P \leq .05$; **$P \leq .01$;***$P \leq .001$). n = 5–6 mice per group.

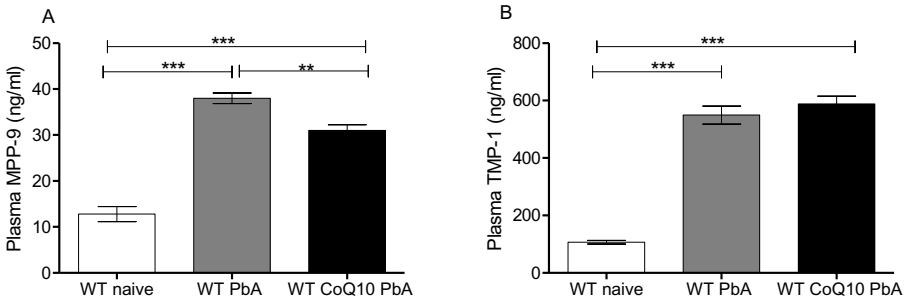

Fig. 4. CoQ_{10} supplementation reduces matrix metalloproteinases in the plasma of PbA-infected mice. C57BL/6J mice were orally supplemented with 200 mg/kg/day of CoQ_{10} for 30 days and simultaneously infected as WT mice with 5×10^4 PbA iRBC. After 6 days of infection, blood was obtained through cardiac puncture and levels of matrix metalloproteinases were determined. (A) Plasma levels of MPP-9 and (B) TMP-1in the plasma were determined by ELISA. Bars shows mean of each group ± SEM and are representative of at least two independent experiments. Matrix metalloproteinases were compared by ANOVA, followed by a Bonferroni posttest (indicated level of significance: $^{**}P \leq .01$;$^{***}P \leq .001$). n = 5–6 mice per group.

Vcam-1 changes in CoQ_{10} treated or untreated PbA infected mice (Fig. 5D).

3.6. Lower levels of Hmox1 mRNA expression in the brains of CoQ_{10} supplemented C57BL/6J mice

Previous literature has shown that expression of Hmox1 occurs during malaria in humans [41], more importantly, incidences of ECM are linked with lower levels of Hmox1 mRNA expression in the brains of C57BL/6 mice [42]. This study sought to determine whether CoQ_{10} supplementation could modulate the expression levels of Hmox1 mRNA in brains of mice following PbA infection. Brain Hmox1 mRNA expression was elevated in CoQ10 supplemented mice infected with PbA as compared to un-supplemented mice (Fig. 6). Therefore we hypothesize that lower HO-1 expression in CoQ_{10} supplemented mice is associated with lower incidence of ECM in C57BL/6 J mice infected with PbA.

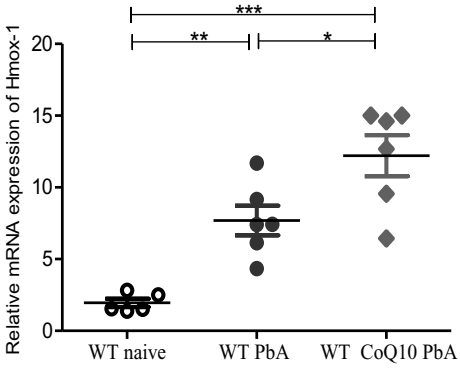

Fig. 6. Increased expression of heme oxygenase-1 among CoQ_{10} supplemented mice during PbA infection. C57BL/6J mice were either orally administered with 200 mg/kg/day with or without CoQ_{10} and were infected with 5×10^4 PbA, iRBC WT mice. Brain cells were collected and assayed for the expression of Hmox-1 by qPCR (relative to β-actin. Results are representative of two independent experiments. Bars represent mean ± SEM. Splenic mRNA Hmox-1 levels were compared by ANOVA, followed by a Bonferroni posttest (indicated level of significance: $^{*}P \leq .05$;$^{**}P \leq .01$; $^{***}P \leq .001$). n = 5–6 mice per group.

3.7. Less pronouncement of Liver pathology in CoQ_{10} supplemented-infected mice

The liver is an important organ involved during the hepatic stage of the malaria parasite's life cycle, where malaria sporozoites develop into merozoites. Previous and current literature has shown that parasites accumulate in many tissues, including the liver, which is one of the sites

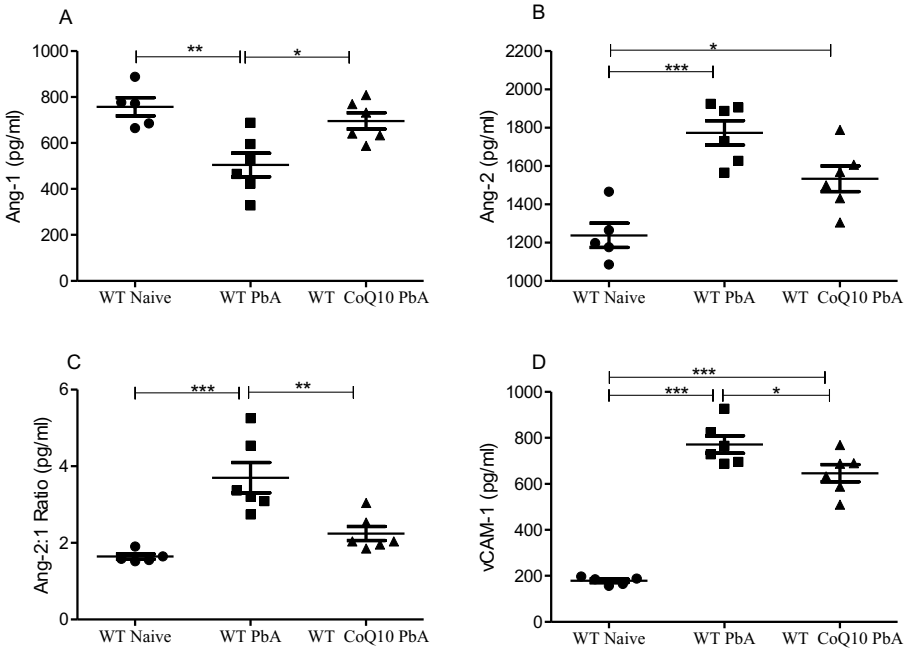

Fig. 5. CoQ_{10} supplemented mice show altered levels of serum angiogenic factors upon PbA infection. Indicated groups of mice were infected i.v. with 5×10^4 iRBC of PbA. Six days later, animals were sacrificed and the blood was drawn from the heart. Serum concentrations of Ang-1 (A), Ang-2 (B), the ratio of Ang-2:Ang-1(C) and Vcam1(D) was measured from uninfected WT, WT PbA infected and CoQ_{10}-PbA infected groups. Results are representatives of three independent experiments. Levels of angiogenic values were compared by ANOVA, followed by a Bonferroni posttest (indicated level of significance: $^{*}P \leq .05$;$^{**}P \leq .01$; $^{***}P \leq .001$. n = 5–6 mice per group.

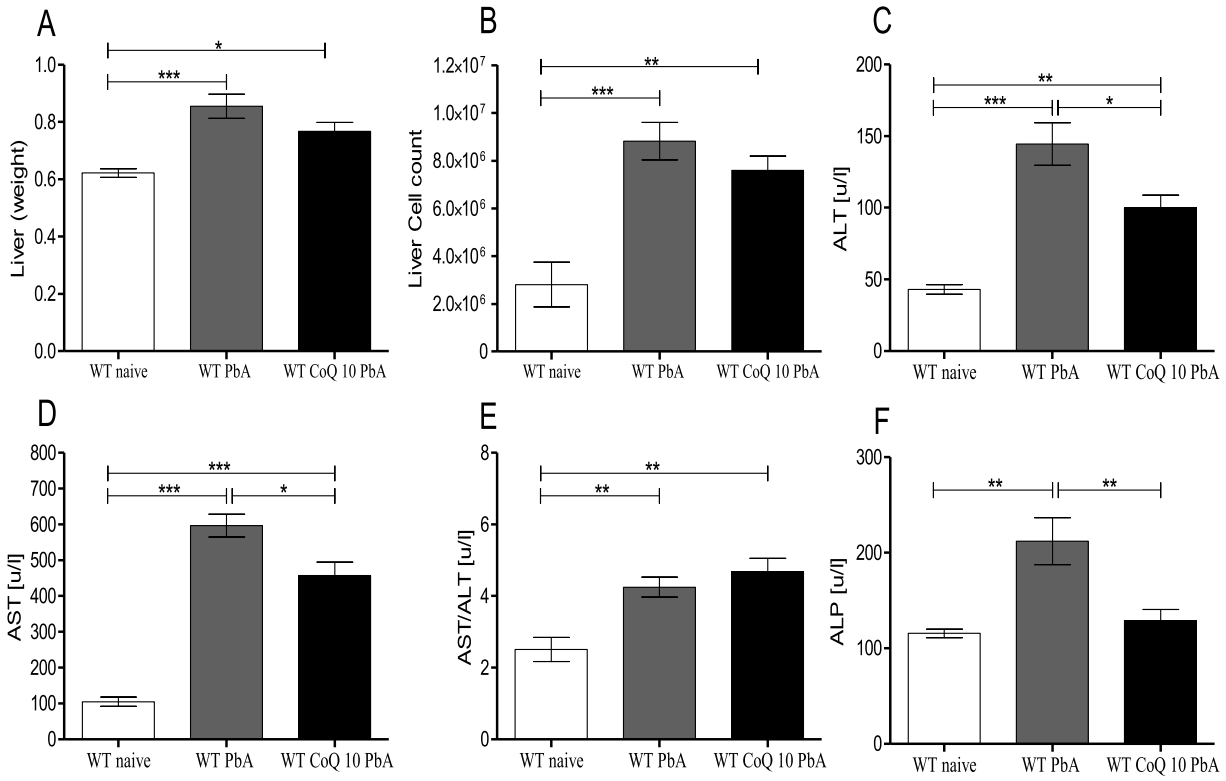

Fig. 7. Impact of CoQ_{10} in the liver during the course of PbA infection.

of greatest parasite tissue sequestration in ECM [43]. Additionally, another study has reported an association between high PRBC load in the livers of malaria patients with jaundice, hepatomegaly and liver enzyme elevation [44]. In order to determine if the protection observed in CoQ_{10} supplemented mice during PbA infection is associated with decreased inflammation in the liver during plasmodium infection, weight, liver associated lymphocyte count and parameters of liver injury were assessed in C57BL/6 mice infected with PbA and supplemented with or without CoQ_{10} at 6 days p.i. Hepatomegaly was more pronounced in PbA infected un-supplemented mice, as indicated by slight decrease in weights in mice orally supplemented with CoQ_{10} at day 6 of ECM (Fig. 7A). At similar time point and after CoQ_{10} administration followed by infection of mice with PbA no major differences were found in the amount of liver associated lymphocytes in this organ between mice supplemented with or without CoQ_{10} (Fig. 7B). Note that high levels of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are an indication of liver pathology. Serum ALT and AST levels decreased at 6 days p.i after infection among CoQ_{10} treated group in comparison to un-supplemented group (Fig. 7C–D) respectively. In contrast, the ratio of ALT and AST was comparable among CoQ_{10}-PbA infected mice and those infected alone (Fig. 7E). This indicates less severe liver pathology development in CoQ_{10}-infected mice than in CoQ_{10} un-supplemented-infected mice, despite similar weight and amount of liver associated lymphocyte levels. Elevated serum levels of alkaline phosphatase (ALP) which has been associated with bile duct damage during malaria was also assessed, however, this enzyme was comparable between CoQ_{10} supplemented and un-supplemented PbA infected mice (Fig. 7F).

Indicated groups of mice were infected i.v. with 5x10e4 iRBC of PbA. Six days later, animals were sacrificed and the blood was drawn from the heart and the Spleens were isolated to prepare single cell suspensions. (A) Liver weight (B), Liver associated lymphocyte count (C), Plasma ALT (D) AST (E) AST:ALT and (F) ALP from uninfected WT, WT PbA infected, CoQ_{10} PbA infected groups. The distribution of these different metabolites in serum was analyzed by fractionation of 30 µL of serum of each mouse. Total metabolite content of the effluent was determined using enzymatic colorimetric assays (Roche Diagnostics). One way ANOVA with Bonfferoni multiple comparisons test, (indicated level of significance: $^{*}P \leq .05$; $^{**}P \leq .01$;$^{***}P \leq .001$). $n = 5$–6 mice per group.

3.8. Serum of CoQ_{10} PbA infected WT mice contain altered levels of lipids

Serum lipid profile changes have been observed during malaria infection with patients exhibiting laboratory abnormalities due to an acute phase response. Several clinical studies have reported that lipid profile changes in the setting of both uncomplicated and complicated malaria [45,46]. Although the magnitude of changes seems to be related to the severity of malaria in several studies [47], others found no correlation between the severity of malaria attacks and the extent of lipid profile changes [48,49]. Therefore, it means that the extent of serum lipid profile changes during malaria infection and their underlying biological mechanisms remain unclear. To this end we measured levels of lipids in our own experimental set-up in the uninfected, WT infected and CoQ_{10} un-supplemented PbA infected mice at 6 days p.i. The WT PbA infected mice had significantly increased total cholesterol and LDL cholesterol levels compared to the CoQ_{10} PbA infected mice (Fig. 8A,C). This observation indicates that low cholesterol and triglycerides levels may be linked to the cellular protection. However, we didn't observe any significant difference in the levels of low density lipoprotein and high density lipoprotein between PbA infected and supplemented with CoQ_{10} and the un-supplemented group (Fig. 8B, D).

3.9. CoQ_{10} supplementation is associated with decreased oxidative stress in the brain tissues of ECM

Indeed, the potential role of oxidative stress during malaria infection remains elusive. Nevertheless, oxidative stress has a direct implication in the exacerbation of neuro-pathogenesis in neurodegenerative disorders and encephalopathy associated with sepsis condition

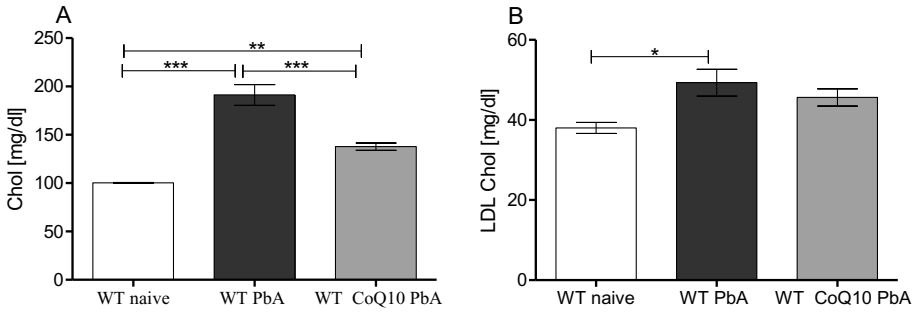

Fig. 8. Comparison of serum lipids in CoQ_{10} supplemented and WT mice enzymes upon PbA infection. Indicated groups of mice were infected i.v. with 5x10e4 iRBC of PbA. Six days later, animals were sacrificed and the blood was drawn from the heart. Serum concentration of: (A) Total cholesterol (B) LDL cholesterol (C) Serum triglyceride and (D) HDL cholesterol from uninfected WT, WT PbA infected and WT CoQ_{10}-PbA infected groups. The distribution of these different metabolites in serum was analyzed by fractionation of 30 µL of serum of each mouse. Total metabolite content of the effluent was determined using enzymatic colorimetric assays (Roche Diagnostics). One way ANOVA with Bonfferoni multiple comparisons test, (indicated level of significance: $^{*}P \leq .05$;$^{***}P \leq .001$). n = 5–6 mice per group.

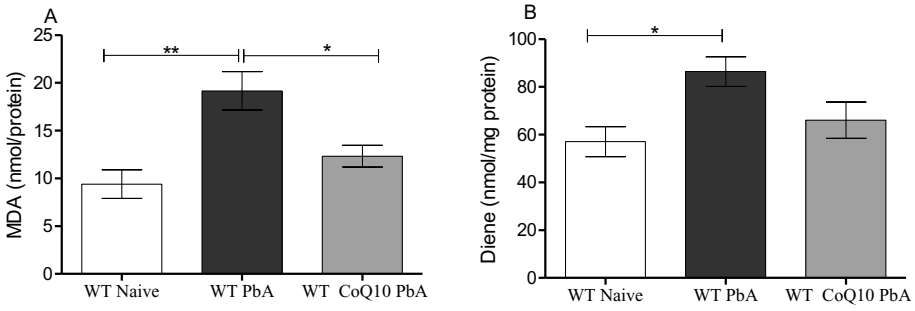

Fig. 9. Oxidative stress is decreased in the brains of mice supplemented with CoQ_{10} during ECM. Indicated groups of mice were infected i.v. with 5x10e4 iRBC of PbA. Six days later, animals were sacrificed, perfused with PBS and the brain harvested. The levels of oxidative stress was determined by measuring conjugated diene (A and B) formation in brains on day 6 of ECM using enzymatic colorimetric assays (Roche Diagnostics). One way ANOVA with Bonfferoni multiple comparisons test, (indicated level of significance: $^{*}P \leq .05$;$^{**}P \leq .01$). n = 5–6 mice per group.

[50,51]. Moreover, researchers have given evidence of the existence of a protective role in malaria, whereas others pin point a relation to the severe pathology of the disease [8]. With that in mind, various studies have shown that oxidative stress directly contribute to lipid peroxidation, promoting functional and structural changes of the plasma membrane that lead to hemolysis; which has always been linked to elevated markers of lipid peroxidation in malaria [52]. However, no research has focused on deciphering the relevance of CoQ_{10} supplementation on attenuating oxidative stress during ECM. Based on this, this study tested whether CoQ_{10} supplementation would modulate lipid peroxidation during plasmodium infection by measuring the generation of MDA, and the formation of diene conjugated species markers of oxidative stress at 6 days post infection. On day 6 post infection, the levels of MDA (Fig. 9A) was decreased in brain tissue for CoQ_{10} supplemented PbA-infected mice when compared to the un-supplemented mice. Conversely, levels of diene conjugates were abrogated in CoQ_{10} supplemented group in comparison to PbA infected mice alone, though not statistically significant (Fig. 9B). These results demonstrate that CoQ_{10} can ameliorate oxidative stress in the brains of C57BL/6 J mice infected with PbA; indicating that oxidative stress is an important component that contributes to the genesis of pathology associated with ECM.

3.10. CoQ_{10} supplementation restores reduced endogenous GSH during PbA infection

As already mentioned the role of oxidative stress in the pathophysiology of malaria is convoluted phenomenon and constitute an indispensable aspect of the sophisticated and complex host-parasite relationship. A number of literatures have demonstrated that generation of reactive oxygen species (ROS) during malaria infection is implicated in the genesis of rheological changes in malaria [53,54]. Whereas initial generation of these ROS is beneficial by defending the host against plasmodium; continuous production of the same results in potential disruption of endogenous antioxidant system. Moreover, the ROS depletes cellular antioxidant defenses such as glutathione (GSH) [55], the GSH antioxidant provides host defense against oxidative stress during Malaria, acting upon several different mechanisms [56]. We hypothesize, that any phenomenon that will compromise the cellular antioxidant system will subject the host to the deleterious effects of oxidative stress and could contribute to severe pathophysiology witnessed in CM. To address this hypothesis, we analyzed the levels of reduced (GSH) and oxidized (GSSG) in the brains of the CoQ_{10} supplemented *versus* un-supplemented mice at 6 days p.i. Importantly, CoQ_{10} supplemented PbA infected mice presented elevated brain total GSH and reduced glutathione compared to PbA infected alone (Fig. 10A–B). In contrast, the levels of oxidized (GSSG) was reduced in the brains of CoQ_{10} supplemented mice (Fig. 10C), whereas the ratio of GSH/GSSG were slightly increased in the brains of CoQ_{10} supplemented mice compared to un-supplemented (Fig. 10D), without reaching a statistical significance. This justifies the improved protection and survival rate of mice administered with CoQ_{10}.

3.11. Low levels of free hemoglobin in plasma of CoQ_{10} supplemented mice contribute to high NO bioavailability during ECM

The fundamental role of nitric oxide (NO) in the exacerbation of

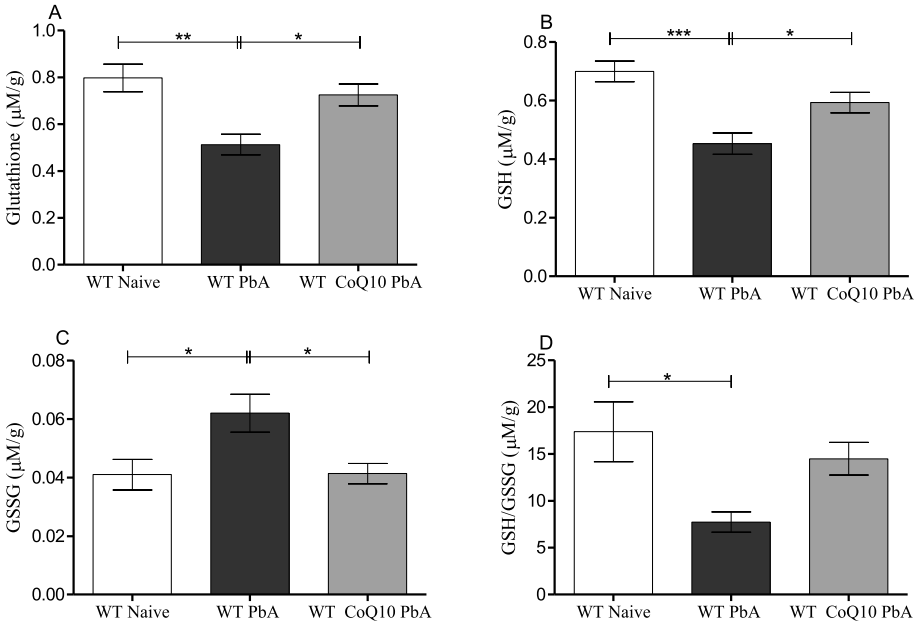

Fig. 10. Comparison of brain glutathione in CoQ_{10} supplemented and WT mice enzymes upon PbA infection. Indicated groups of mice were infected i.v. with 5x10e4 iRBC of PbAma1. Six days later, animals were sacrificed and the brain was extracted. Brain concentration of: (A) Total glutathione (B) Reduced glutathione (C) oxidized glutathione and (D) GSH:GSSG ratio from uninfected WT, WT PbA infected and WT CoQ_{10}-PbA infected groups. The distribution of this endogenous antioxidant in brain was analyzed by fractionation of brain homogenate of each mouse. Glutathione content of the effluent was determined using enzymatic colorimetric assays (Roche Diagnostics). One way ANOVA with Bonfferoni multiple comparisons test, (indicated level of significance: $*P \leq .05$; $**P \leq .01$; $***P \leq .001$). n = 5–6 mice per group.

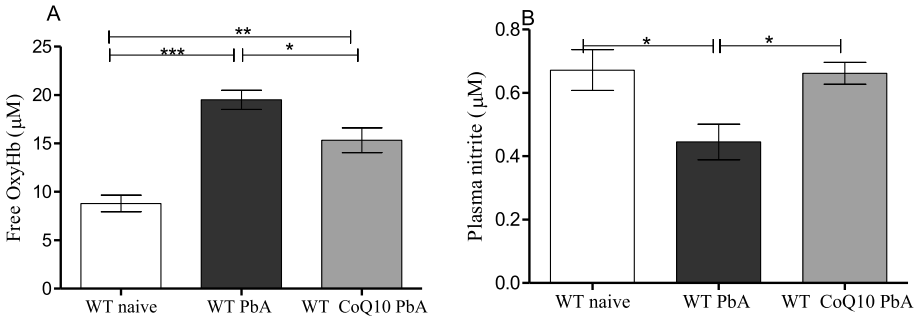

Fig. 11. CoQ_{10} supplemented mice contain low levels of Free hemoglobin in plasma and high levels of plasma NO during ECM. C57BL/6 J mice were either orally administered with or without 200 mg/kg/day CoQ_{10} and were infected with 5×10^4 PbA iRBC. Levels of Free Oxyhemoglobin (*A*) and plasma nitrite (B) from plasma in the control groups and in PbA iRBC-infected wt and CoQ10 PbA infected mice at d + 6 p.i. Levels of free oxyhemoglobin were quantified spectrophotometrically at 578 nm while levels of NO were determined by Griess assay. The bars show the mean of each group ± SEM. Levels of free oxyhemoglobin and NO was compared by ANOVA, followed by a Bonferroni posttest (indicated level of significance: $*P \leq .05$; $**P \leq .01$;$***P \leq .001$). n = 5–6 mice per group.

cerebral malaria remains contentious. Nevertheless, it has been demonstrated recently that low NO availability is associated with severe malaria in murine model [57]. To determine how levels of nitric oxide vary in plasma of mice supplemented with or without CoQ_{10} during PbA infection, we used Grissy assay to assess the concentration of nitrites in plasma on day 6 of ECM. Concentrations of nitrite were significantly higher in plasma of CoQ10 supplemented mice on day 6 of ECM compared with WT PbA infected mice alone (Fig. 11B). These data support the hypothesis that high levels of NO bioavailability results in amelioration of ECM. During ECM, a number of hypothesis have been advanced to illustrate the probable causes that contributes to abrogation of NO both in plasma and in erythrocytes, which include scavenging of NO by free oxyhemoglobin, hypoargininemia and low levels of nitrite which is usually converted by deoxyhemoglobin to NO [58,59]. To examine this issue in CoQ_{10} supplementation during ECM, levels of free oxyhemoglobin in plasma was measured on day 6 p.i. It was noted that the concentration of free oxyhemoglobin levels was significantly low in mice supplemented with CoQ_{10} on day 6 p.i as compared to un-supplemented mice (Fig. 11A). A decrease in the levels of free hemoglobin in the plasma of CoQ_{10} supplemented mice is an indicative of high NO bioavailability; observed in these mice during ECM.

3.12. Mice supplemented with CoQ_{10} had reduced levels of mitochondrial 8-OHdG in the brains during ECM

It has been reported that increase in levels of 8-hydroxy-2-deoxyguanosine (8-OHdG), which is a marker of oxidative DNA damage correlate with pathogenesis of diabetic nephropathy [60]. Moreover, other studies have revealed that high levels of 8-OHdG are associated with cardiovascular disease [61]. Additionally, using cancer tissues from the colon, Płachetka and colleagues have shown that the level of 8-OHdG is directly linked to the severity of colorectal adenocarcinoma [62]. Based on these evidences, the effect of plasmodium infection in accentuating DNA damage during ECM and with concomitant attenuation of the same with CoQ_{10} administration was explored. Therefore, both nuclear and mitochondria DNA (nDNA & mtDNA) was extracted from brains of mice with or without CoQ_{10} supplementation at day 6 of ECM. Concentration level of 8-OHdG was measured by ELISA. The levels of 8-OHdG in the mtDNA were significantly abrogated in the brains of CoQ_{10} supplemented mice as compared with those from the un-supplemented PbA infected at day 6 of ECM (Fig. 12A). By contrast, the levels of 8-OHdG in nuclear DNA were comparable across the groups (Fig. 12B).

3.13. CoQ_{10} supplementation prevents the effects of PbA infection on NF-κB signaling pathway

In severe malaria, NF-κB has been implicated in enhancing production of inflammatory cytokines [63]. Additionally, NF-κB p65 activation and apoptosis is associated with the progression of cerebral malaria [64]. Importantly, antioxidants can modulate components of stress induced apoptotic pathways, protein kinase SAPK/JNK and

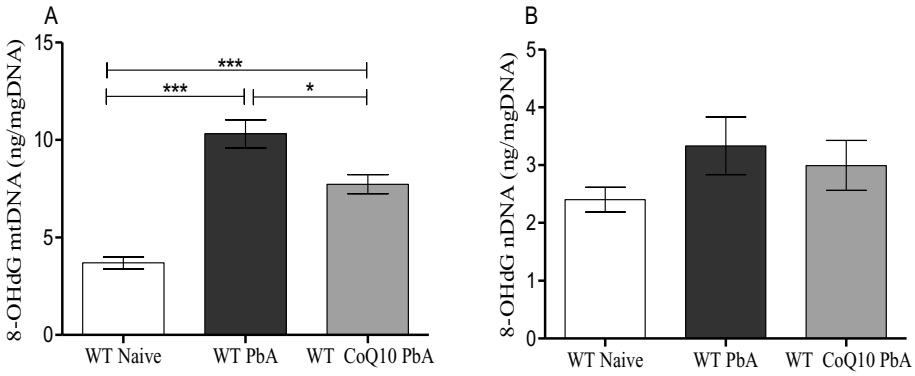

Fig. 12. CoQ_{10} supplemented mice contain low levels of of 8-OHdG in mtDNA in the brain upon PbA infection. C57BL/6 J mice were either orally administered with or without 200 mg/kg/day CoQ10 and were infected with 5×10^4 PbA iRBC. Levels of 8-OHdG in mtDNA (A) and nuclear DNA (B) from brains in the control groups and in PbA iRBC-infected wt and CoQ_{10} PbA infected mice at d + 6 p.i. mtDNA and nDNA were extracted separately from brains of mice. Levels of 8-OHdG were quantified by ELISA kit. Bars shows mean of each group ± SEM. The level of 8-OHdG in mtDNA and nDNA was compared by ANOVA, followed by a Bonferroni posttest (indicated level of significance: $*P \leq .05$;$***P \leq .001$). $n = 5–6$ mice per group.

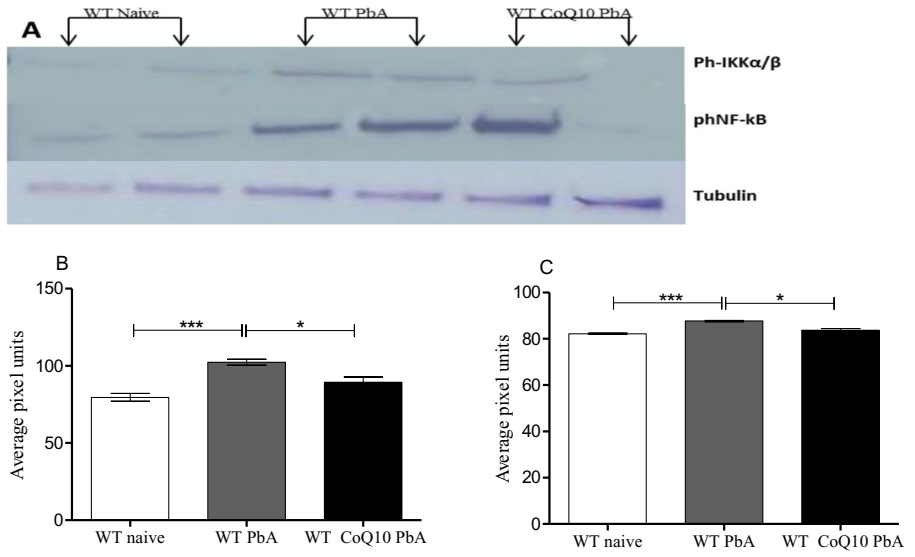

Fig. 13. Quantitative analysis of IKKα/β and ph NF-κB phosphorylation signal proteins in the brains of WT Naive, WT-PbA and WT-CoQ_{10}-PbA infected mice. C57BL/6 J mice were either orally administered with or without 200 mg/kg/day CoQ_{10} and were infected with 5×10^4 PbA iRBC. Immuno-blot of nitrocellulose membranes showing specific expression of IKKα/β and ph NF-κB signal protein that was determined in brain homogenate fractions (A). The histogram shows semi-quantitative determinations of ph NF-Kb and of IKKα/β protein expression (A-B) presented as percentage pixel intensity. Levels of signal proteins from brain homogenates were analyzed in the control groups and in PbA iRBC-infected wt and CoQ10 PbA infected mice at d + 6 p.i. Bars shows mean of each group ± SEM. Levels of ph NF-Kb and of IKKα/β protein exprwas compared by ANOVA, followed by a Bonferroni posttest (indicated level of significance: $*P \leq .05$;$***P \leq .001$). $n = 5–6$ mice per group.

transcription factor NF-κB thereby contributing to reduction in the inflammatory responses [65]. Considering all these facts, it is probable that NF-κB signaling has direct correlation to a variety of immune-pathological processes. On this basis, the antioxidant and modulatory activity of CoQ_{10} in the brain during ECM was tested. Therefore, in order to explore the plausible mechanisms of CoQ_{10} in response to plasmodium generated toxic stress; the action of NF-κB cascade pathway was determined by quantifying the IKKα/β and ph NF-κB phosphorylation signal proteins by western blot. Strikingly, mice supplemented with CoQ10 presented significantly low levels of IKKα/β and ph NF-κB in the brain at day 6 of ECM (Fig. 13B–C), indicating the ability of CoQ_{10} to reduce NF-κB pathway activation under PbA induced acute toxic stress.

4. Discussion

In this study we demonstrate that Coenzyme Q_{10} has the capacity to ameliorate oxidative stress and inflammation in PbA infected mice ensuing in protection against experimental cerebral malaria. Heightened augmentation of oxidative stress has been associated with pathological mechanisms of CM [11]. Numerous attempts have been made previously to lessen levels of oxidative stress during cerebral malaria; specifically it was shown that transient supplementation of SOD-1 which is an endogenous antioxidant protects against *P. falciparum* induced oxidative stress and apoptosis [18]. Coenzyme Q_{10} is an essential cofactor in the bioenergetics reactions and a powerful antioxidant that acts against oxidative stress [66,67], and a regulator of gene expression [68]. These roles has resulted in Coenzyme Q_{10} being investigated for treatment of neurodegenerative disorders, diabetes mellitus, and cardiovascular diseases [69–71]. Despite this remarkable observation, there exists no data on the role of CoQ_{10} during ECM. Therefore, in the current study it was hypothesized that CoQ_{10} might reduce oxidative stress and inflammation during PbA infection in mice.

In the present study, infected animals supplemented with CoQ_{10} had improved survival rates. In fact 100% of the wild type mice presented characteristic symptoms associated with cerebral malaria and invariably died rapidly in a time phase of 6–9 days. On the other hand, CoQ_{10} administered mice were significantly protected with 62.5% of mice surviving past the ECM phase (6–8 days). The improved survival rate in CoQ_{10} group might be attributed to the antioxidant ability of CoQ_{10} to delay the establishment of mechanisms that lead to neuropathology. Therefore, these results indicate that despite the fact that antioxidant therapy has limited clinical applications; oral administration of Co-Q_{10} prevented lethal ECM in majority of mice infected with PbA in the current study. This is a remarkable finding to say the least.

As a widely accepted model of studying cerebral malaria, C57BL/6J develops oedema as one of the neurological features associated with severe malaria [72]. Previously it has been reported that oedema is more pronounced in mice suffering from severe malaria and the level correlates with neuropathology of the diseases [73]. Studies from both human and mouse model showed that when the integrity of the blood-brain barrier is compromised it leads to severe brain oedema [74]. Consequently, the formation of brain oedema aggravates hypoxia by constricting cerebral blood vessels, resulting in blood flow blockage and ultimately death in mice [35]. Herein, the development of oedema in PbA infected mice was abrogated by administration of exogenous

CoQ_{10}. The role of CoQ_{10} to counteract brain Oedema during ECM merit further investigations.

In a systematic review and meta-analysis, it was putatively demonstrated that exogenous administration of CoQ_{10} significantly decreased genes for inflammation especially TNF-α levels [75]. A previous study working with CoQ_{10} reported down-regulation of inflammatory markers associated with multiple sclerosis [76]. Additionally, *in vitro* studies demonstrated that CoQ_{10} is a modulator of inflammatory gene expression [77]. Therefore, in view of the prominent inflammatory responses and antioxidant system failure due to plasmodium infections, employing compounds known to possess potent antioxidant and anti-inflammatory properties in ameliorating severity of the disease seemed logical. In the current investigation, augmentation of brain TNF-α and MIP-1β inflammatory genes in PbA-infected mice was not surprising. Interestingly, CoQ_{10} administered PbA infected mice attenuated these inflammatory genes indicative of less inflammation in the brain of these mice.

Matrix metalloproteinases (MMPs) are class of proteolytic enzymes which are involved in regulation of inflammatory responses and disruption of endothelial tight junctions. A large body of growing evidence from both human and animal studies has implicated MMPs as major players in the genesis of malaria [78,79]. Previously, it was shown that MMPs are involved in the disruption of blood-brain barrier (BBB) and pathology observed in cerebral malaria [80]. Particularly, MMP-9 has been implicated in the destruction of BBB in a number of diseases and is also involved in the shedding of membrane bound TNF-α [81–84]. Given the critical role of MMPs in exacerbation of the neuropathology in CM, down regulation of these MMPs might improve the outcome of ECM. Importantly, oral administration of PbA- infected mice with CoQ_{10} potently decreased production of MMP-9. These results highlight the impact of CoQ_{10} on production and expression of MMPs during ECM.

It is widely accepted that during the pathogenesis of CM, endothelial cell system activation and dysfunction takes place in the brain [85]. Overproduction of activated endothelial markers are significantly increased in malaria and directly correlated with severity of the disease [86–88]. However, it is not clear whether endothelial markers are important as predictor markers for assessment of disease progression or outcome. Nevertheless, Angiopoietins which are endothelial protein markers have been shown to play a critical physiological role in maintenance of vascular integrity. More importantly in the context of malaria, serum Angiopoietin-1 (ANG-1) levels are normally low while Angiopoietin-2 (ANG-2) levels are very high [89]. Consequently, increase in ANG-2 and dysregulation of ANG-1/2 ratio is associated with disease severity and is a predictor of CM [90]. The usefulness of CoQ_{10} administration to improve endothelial dysfunction has been documented in cardiovascular diseases [91,92]. Our results provides further compelling evidence to demonstrate that CoQ_{10} administration maintained the normal levels of ANG-1 and ANG-1/2 balance, that favors the host protection against the deleterious effects of CM. This key observation can be attributed to the beneficial aspect of CoQ_{10} to improve the endothelial system owing to it is antioxidant, anti-inflammatory and improvement of NO bioavailability [91,92].

Release of free heme during Plasmodium infection has been associated with destruction of the BBB and enhancement of ECM pathogenesis [42]. Heme-oxygenase −1(HO-1) is a vital enzyme that is produced during malaria and is essential for conversion of heme to CO, iron and biliverdin [93]. Generation of CO by HO-1 has been shown to protect mice against ECM by reducing accumulation of adhesion molecules in the brain microvasculature and infiltration of leukocytes into the brain thereby abrogating BBB disruption ([42] In PbA-infected mice, CoQ_{10} administration induced expression of HO-1 in the brains of this mice, an indicative of ECM protection. Therefore, the protection of CoQ_{10} against ECM in this context can be linked to the ability of CoQ_{10} not only to induce expression of HO-1 but also to reduce peroxide mediated toxicity as well as free heme associated toxicity. Indeed, accumulating evidence supports our observation; particularly administration of curcumin during ECM enhanced upregulation of HO-1 that possesses both pro and anti-oxidant activities. Hence curcumin has the capacity to reduce heme toxicity and ROS [94].

It is known that increased inflammation in the liver is associated with elevated levels of aminotransferases enzyme in the serum [95]. Moreover, it has been demonstrated that malaria parasites accumulate in many tissues, including the liver, which is one of the sites of greatest parasite tissue sequestration in ECM [43]. Additionally, another study has reported an association between high PRBC load in the livers of malaria patients with jaundice, hepatomegaly and liver enzyme elevation [44]. Thus, liver damage was analyzed during PbA-infection and whether administration of CoQ_{10} can ameliorate the same. Here we show that CoQ_{10} administration was able to decelerate liver pathology as shown by significant decrease in liver aminotransferases enzymes that are associated with pathology.

Malaria parasites are usually in a constant demand for lipids from the host for their growth [96]. Current studies have shown that total cholesterol and triglycerides are significantly increased during ECM [73]. Previously, it has also been demonstrated that serum cholesterol and triglycerides are enhanced in mice suffering from ECM compared to the protected mice [97]. Coenzyme Q_{10} has been credited with lipid lowering effects, especially for cholesterol and triglycerides in serum thereby ameliorating obesity in mice [98]. Hence the altered cholesterol and triglycerides in serum samples of CoQ_{10} administered mice on day 6 of ECM could reflect many aspects that may be related to protection. Changes in serum lipid profile are also associated with anaemic conditions during malaria. Particularly, in adult Indians suffering from iron deficiency anaemia, levels of triglycerides were found to be highly augmented in there serum [99]. In the present study anaemia was detected in PbA-infected mice on day 6 of ECM as reflected by low levels of PCV (Haematocrit) whereas in CoQ_{10} administered mice anaemia was abrogated. It is therefore possible that serum lipid variations already observed can be linked with anaemia during periods of ECM.

Generation of oxidative stress has a direct implication in the exacerbation of neuro-pathogenesis in neurodegenerative disorders and encephalopathy associated with sepsis condition [50,51]. Moreover, researchers have given evidence of the existence of a protective role in malaria, whereas others pin point a relation to the severe pathology of the disease [8]. Nevertheless, oxidative stress directly contribute to lipid peroxidation, promoting functional and structural changes of the plasma membrane that lead to hemolysis, which has always been linked to elevated markers of lipid peroxidation in malaria [52]. Specifically, [12] clearly demonstrated that lipid peroxidation markers were highly elevated during ECM, and a significant increase in oxidative stress in the brains of PbA infected mice was ameliorated by additive antioxidant therapy. Our findings indicate that oral administration of CoQ_{10} was able to attenuate lipid peroxidation on day 6 post-infection in mice. This data is consistence with our previous results that showed CoQ_{10} can ameliorate oxidative stress during Human African Trypanosomiasis [24]. CoQ_{10} scavenges free radicals and enhances membrane integrity by preventing oxidation of lipids within biological membranes [20]. Since lipid oxidation is correlated with membrane disintegration and ultimate cell death [100], it is possible that CoQ_{10} protected animals against lipid peroxidation by keeping levels of free radicals in the blood low; thereby shielding cell membranes against oxidative damage. Moreover, the usefulness of CoQ_{10} to attenuate lipid peroxidation is based on its intricate interaction during the peroxidation process. CoQ_{10} primarily acts by preventing generation of lipid peroxyl radicals, leading to reduction in the initiation of perferryl radical with the formation of ubisemiquinone and H_2O_2.

Oxidative stress, which predominantly targets brain endothelial cells, can also participates in the pathogenesis usually witnessed in ECM. Previous findings have indicated that ECM is associated with depletion of the brain glutathione pool presumably due to the marked oxidative stress with concomitant utilization of GSH [101]. Findings in

this study indicate that PbA-infected mice showed a decrease in the brain levels of GSH, whereas oral administration of CoQ_{10} during ECM restored GSH levels. Such effect by CoQ_{10} suggests that this powerful antioxidant had a sparing effect on PbA-infected mice endogenous antioxidants. Therefore, the decrease in brain glutathione pool during PbA-infection can be particularly an indicator of disease severity. Nevertheless, levels of SOD and glutathione have been reported to be decreased in children suffering from severe malaria [13]. Equally, depletion of brain GSH during PbA-infection has been observed in mice [101], demonstrating that infection can perturb the antioxidant defenses and the redox buffering system in the brain [102], which in turn impairs their functions with putative resultant neurological dysfunction generally, observed in the ECM pathogenesis. The effect of Oral administration of CoQ_{10} in restoring GSH was likely due to scavenging of ROS or by activating antioxidant response elements upstream of genes that are key mediators in detoxification and anti-oxidation. Since GSH reduction has been reported in patients and animal models of various neurodegenerative disorders such as Parkinson's and Alzheimer's disease [103,104]), this phenomenon could play a major role in the induction of CM pathogenesis.

Overall, oxidative stress plays a key role in oxidative damage of cellular macromolecules within the host. Increasing evidence suggest that mtDNA is 20 fold more susceptible to mutation and oxidative damage than nDNA [105,106]. Oxidative stress due to disproportion flanked by the generation of free radicals and reduction in antioxidant capacity, induces oxidation of deoxyguanosine to 8-hydroxy-2-deoxyguanosine (8-OHdG), followed by mutation of mtDNA, which has continued to receive attention in recent years. Particularly, it has been shown that oxidative DNA damage correlate with pathogenesis of diabetic nephropathy [60]. Other studies have revealed that high levels of 8-OHdG are associated with cardiovascular disease [61]. Additionally, using cancer tissues from the colon, Płachetka and colleagues have shown the levels of 8-OHdG is directly linked to the severity of colorectal adenocarcinoma [62]. By measuring the concentration levels of 8-OHdG on day 6 of ECM, we found out that CoQ_{10} significantly abrogated levels of 8-OHdG brain mtDNA. Since CoQ_{10} acts by quenching free radicals thereby keeping oxidative stress in check, it must be critically important for counter-regulating the oxidative damage of mtDNA. This protective mechanism is therefore an important component of cellular defense against ROS and other free radicals in varied situations in the cell.

The severity of ECM is also characterized by low levels of NO bioavailability [107]. Similarly, development of CM in Tanzanian children is associated with low levels of nitrites as well as small amount of plasma levels of L-arginine [108,109]. Probable causes leading to diminished NO bioavailability during ECM is due to upsurge in free hemoglobin that usually scavenge for NO, low nitrite and hypoargininemia [110]. We found that plasma from PbA-infected mice presented a high free oxyhemoglobin and low levels of NO. Co-enzyme Q_{10} was able to protect animals against the observed decline in NO levels and significantly increase free oxyhemoglobin following on day 6 of ECM. Suprisingly CoQ_{10} has been shown to suppress production of nitrite promoted by TNF-α in mice induced with dermatitis [111]. In fact, our findings indicate the converse: explicitly showing that CoQ_{10} administration restores NO bioavailability that is associated with a protective mechanism during ECM. It is therefore hypothesized that CoQ_{10} administration might abrogate the concentration of free oxyhemoglobin in the circulation by enabling it to converted to methemoglobin (NO-inert) thereby allowing endogenous NO to fulfil its primary homeostatic function.

NF-kB (nuclear factor k beta) plays a key role in immune and inflammatory responses in Malaria; specifically generation of pro-inflammatory cytokines that is strictly interrelated to NF-κB protein transcription activation [63]. Growing evidence from *in vitro* studies has demonstrated that monocytes and macrophages can be stimulated by haemozoin and glycosylphosphatidylinositol to produce proinflammatory cytokines and chemokines through NF-κB downstream signaling pathways [112–114]. Such enhanced production of pro-inflammatory cytokines can be associated with acute exacerbation of the pathogenesis of malaria. Additionally, phosphorylation of NF-κB components is an eccentric stage warranting translocation of NF-κB into the nucleus to initiate the process of gene expression. More importantly, Coenzyme Q_{10} plays an important role by inhibiting NF-κB activation thereby nullifying production of inflammatory genes [115–117]. Considering all these facts, it seems that NF-κB signaling has direct correlation to a variety of immunopathological processes. Indeed, we found that CoQ_{10} was more effective in decreasing NF-κB phosphorylation at 6 days post infection. Thus, it seems that CoQ_{10} can exert its effect by mediating gene expression under PbA induced acute toxic stress. Therefore, these results are consistence with the effect of CoQ_{10} on gene expression described elsewhere [117]. Nevertheless, NF-κB pathway has been shown to be inhibited by a number of compounds that possesses anti-oxidant activities [118–119]. The possibility that CoQ_{10} might be mediating its effect directly by reducing the influx of inflammatory cells in the brain with concomitant reduction in inflammatory parameters associated with the infection and ECM complication cannot be overruled.

In conclusion, we demonstrate that oral CoQ_{10} administration prevented the occurrence of oxidative stress and inflammation in ECM during PbA infection in mice. The observed phenomenon from this study provides further compelling evidence for the role of antioxidant failure in CNS pathology due to *Plasmodium i*nfections. The protective effect of CoQ_{10} administration is consistent with its role as a powerful antioxidant against generation of free radicals, dampening of genes associated with inflammation and restoration of endogenous antioxidant system. These findings demonstrate the potential for CoQ_{10} antioxidant intervention to reverse deleterious phenotypes of ECM developmental programming and therefore provide insight into a potential translatable therapy. However, the efficacy of CoQ_{10} against human malaria will require future testing in clinical studies either alone or in combination with antimalarial regimens, which is the current standard of care for CM.

Conflicts of interest statement

We declare that is no any conflict of interest arising from this work.

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References

[1] WHO, World Malaria Report 2016, WHO Global Malaria Programme, 2017.
[2] L. Schofield, G.E. Grau, Immunological processes in malaria pathogenesis, Nat. Rev. Immunol. 5 (2005) 722–735, https://doi.org/10.1038/nri1686.
[3] R. Idro, K. Marsh, C.C. John, C.R. Newton, Cerebral malaria: mechanisms of brain injury and strategies for improved neurocognitive outcome, Pediatr. Res. 68 (2010) 267–274, https://doi.org/10.1203/00006450-201011001-00524.
[4] N.H. Hunt, J. Golenser, T. Chan-Ling, S. Parekh, C. Rae, S. Potter, I.M. Medana, J. Miu, H.J. Ball, Immunopathogenesis of cerebral malaria, Int. J. Parasitol. 36 (2006) 569–582, https://doi.org/10.1016/j.ijpara.2006.02.016.
[5] C. Engwerda, E. Belnoue, A.C. Gruner, L. Renia, Experimental models of cerebral malaria, Curr. Top. Microbiol. Immunol. 297 (2005) 103–143.
[6] C. Li, E. Seixas, J. Langhorne, Rodent malarias: the mouse as a model for understanding immune responses and pathology induced by the erythrocytic stages of the parasite, Med. Microbiol. Immunol. 189 (2001) 115–126, https://doi.org/10.1007/s430-001-8017-8.
[7] S. de Kossodo, G.E. Grau, Profiles of cytokine production in relation with

susceptibility to cerebral malaria, J. Immunol. 151 (1993) 4811–4820.
[8] M. Sohail, A. Kaul, M. Raziuddin, T. Adak, Decreased glutathione-*S*-transferase activity: diagnostic and protective role in *vivax* malaria, Clin. Biochem. 40 (2007) 377–382.
[9] M. Guha, S. Kumar, V. Choubey, P. Maity, U. Bandyopadhyay, Apoptosis in liver during malaria: role of oxidative stress and implication of mitochondrial pathway, FASEB J. 20 (2006) 439–449.
[10] P. Sobolewski, I. Gramaglia, J.A. Frangos, M. Intaglietta, H.V.D. Heyde, *Plasmodium berghei* resists killing by reactive oxygen species, Infect. Immun. 73 (2005) 6704–6710.
[11] T.L. Souza, A.C.B. Grauncke, L.R. Ribeiro, F.K. Mello, S.M. Oliveira, F. Brant, F.S. Machado, M.S. Oliveir, Cerebral malaria causes enduring behavioral and molecular changes in mice brain without causing gross histopathological damage, Neuroscience. 369 (2018) 66–75.
[12] P.A. Reis, V. Estato, T.I. da Silva, J.C. d'Avila, L.D. Siqueira, E.F. Assis, P.T. Bozza, F.A. Bozza, E.V. Tibirica, G.A. Zimmerman, H.C. Castro-Faria-Neto, Statins decrease neuroinflammation and prevent cognitive impairment after cerebral malaria, PLoS Pathog. 8 (762) (2012) e100099.
[13] N. Narsaria, C. Mohanty, B.K. Das, S.P. Mishra, R. Prasad, Oxidative stress in children with severe malaria, J. Trop. Pediatr. 58 (2012) 147–150.
[14] M. Patel, Targeting oxidative stress in central nervous system disorders, Trends Pharmacol. Sci. 37 (2016) 768–778.
[15] R. Dringen, P.G. Pawlowski, J. Hirrlinger, Peroxide detoxification by brain cells, J. Neurosci. Res. 79 (2005) 157–165.
[16] M.A. Mubaraki, T.A. Hafiz, S. Al-Quraishy, M.A. Dkhil, Oxidative stress and genes regulation of cerebral malaria upon *Zizyphus spina-christi* treatment in a murine model, Microb. Pathog. 107 (2017) 69–74.
[17] T. Imai, T. Iwawaki, R. Akai, K. Suzue, M. Hirai, T. Taniguchi, H. Okada, H. Hisaeda, Evaluating experimental cerebral malaria using oxidative stress indicator OKD48 mice, Int. J. Parasitol. 44 (2014) 681–685.
[18] Z. Taoufiq, P. Pino, N. Dugas, M. Conti, M. Tefit, D. Mazier, I. Vouldoukis, Transient supplementation of superoxide dismutase protects endothelial cells against *Plasmodium falciparum*-induced oxidative stress, Mol. Biochem. Parasitol. 150 (2006) 166–173.
[19] S. Kehr, N. Sturm, S. Rahlfs, J.M. Przyborski, K. Becker, Compartmentation of redox metabolism in malaria parasites, PLoS Pathog. 6 (2010) e1001242.
[20] M. Spindler, M.F. Beal, H. Claire, Coenzyme Q_{10} effects in neurodegenerative disease, Neuropsychiatr. Dis. Treat. 5 (2009) 597–610.
[21] S. McCarthy, M. Somayajulu, M. Sikorska, H. Borowy-Borowski, S. Pandey, Paraquat induces oxidative stress and neuronal cell death; neuroprotection by water-soluble Coenzyme Q10, Toxicol. Appl. Pharmacol. 201 (2004) 21–31.
[22] K. Winkler-Stuck, F.R. Wiedemann, C.W. Wallesch, W.S. Kunz, Effect of coenzyme Q10 on the mitochondrial function of skin fibroblasts from Parkinson patients, J. Neurosci. 220 (2004) 41–48.
[23] J.N. Nyariki, J.K. Thuita, A.M. Wambugu, N.O. Nyamweya, K. Rashid, G.K. Nyambati, A.O. Isaac, Coenzyme Q10 and endogenous antioxidants neuroprotect mice brain against deleterious effects of melarsoprol and *Trypanasoma brucei rhodesiense*, J. Nat. Sci. Res. 8 (2018) 61–73.
[24] K. Rashid, F.N. Wachira, J.N. Nyariki, B. Wanyonyi, G. Murilla, A.O. Isaac, Kenyan purple tea anthocyanins ability to cross the blood brain barrier and reinforce brain antioxidant capacity in mice, Nutr. Neurosci. 17 (2014) 179–185.
[25] C. Schmelzer, G. Lorenz, G. Rimbach, F. Doring, In Vitro effects of the reduced form of coenzyme Q10 on secretion levels of TNF alpha and chemokines in response to LPS in the human monocytic cell line THP-1, J. Clin. Biochem. Nutr. 44 (2007) 62–66.
[26] C. Kilkenny, W.J. Browne, I.C. Cuthill, M. Emerson, D.G. Altman, Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research, PLoS Biol. 8 (2010) e1000412.
[27] R.J. Lundie, T.F. de Koning-Ward, G.M. Davey, C.Q. Nie, D.S. Hansen, L.S. Lau, J.D. Mintern, G.T. Belz, L. Schofield, F.R. Carbone, J.A. Villadangos, B.S. Crabb, W.R. Heath, Blood-stage Plasmodium infection induces CD8+ T lymphocytes to parasite-expressed antigens, largely regulated by CD8alpha+ dendritic cells, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 14509–14514.
[28] N.N. Haj-Yasein, G.F. Vindedal, M. Eilert-Olsen, G.A. Gundersen, Ø. Skare, P. Laake, A. Klungland, A.E. Thorén, J.M. Burkhardt, O.P. Ottersen, E.A. Nagelhus, Glial-conditional deletion of aquaporin-4 (Aqp4) reduces blood-brain water uptake and confers barrier function on perivascular astrocyte end feet, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 17815–17820.
[29] P.T.K. Woo, The haematocrit centrifuge technique for the diagnosis of African trypanosomiasis, Acta Trop. 27 (1970) 384–386.
[30] S.E. Kahn, B.F. Watkins, E.W. Bermes, An evaluation of a spectrophotometric scanning technique for measurement of plasma hemoglobin, Ann. Clin. Lab. Sci. 11 (1981) 126–131.
[31] O.W. Griffith, Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine, Anal. Biochem. 106 (1980) 207–212.
[32] M.W. Pfaffl, A new mathematical model for relative quantification in real-time RT-PCR, Nucleic Acids Res. 29 (2001) 45.
[33] H.H. Draper, M. Hadley, Malondialdehyde determination as index of lipid peroxidation, Methods Enzymol. 186 (1990) 421–431.
[34] H. Puhl, G. Waeg, H. Esterbauer, Methods to determine oxidation of low density lipoproteins, Methods Enzymol. 233 (1994) 425–441.
[35] M.F. Penet, A. Viola, S. Confort-Gouny, Y. Le Fur, G. Duhamel, F. Kober, D. Ibarrola, M. Izquierdo, N. Coltel, B. Gharib, G.E. Grau, P.J. Cozzone, Imaging experimental cerebral malaria *In vivo*: significant role of ischemic brain edema, J. Neurosci. 25 (2005) 7352–7358.
[36] L.A. Sanni, The role of cerebral oedema in the pathogenesis of cerebral malaria, Redox Rep. 6 (2001) 137–142.
[37] A. Szklarczyk, M. Stins, E.A. Milward, H. Ryu, C. Fitzsimmons, D. Sullivan, K. Conant, Glial activation and matrix metalloproteinase release in cerebral malaria, J. Neurovirol. 13 (2007) 2–10.
[38] A. Dietmann, R. Helbok, P. Lackner, S. Issifou, B. Lell, P.B. Matsiegui, M. Reindl, E. Schmutzhard, P.G. Kremsner, Matrix metalloproteinases and their tissue inhibitors (TIMPs) in Plasmodium falciparum malaria: serum levels of TIMP-1 are associated with disease severity, J. Infect. Dis. 197 (2008) 1614–1620.
[39] M.D. Gerdie, J.S. Jasper, V.J. Annelies, van H, Perry JJ., Systematic review of the role of angiopoietin-1 and angiopoietin-2 in *Plasmodium* species infections: biomarkers or therapeutic targets? Malar. J. 15 (2016) 581.
[40] H.J. Schluesener, P.G. Kremsner, R. Meyermann, Heme oxygenase-1 in lesions of human cerebral malaria, Acta Neuropathol. 101 (2001) 65–68.
[41] A. Pamplona, A. Ferreira, J. Balla, V. Jeney, G. Balla, S. Epiphanio, A. Chora, C.D. Rodrigues, I.P. Gregoire, M. Cunha-Rodrigues, S. Portugal, M.P. Soares, M.M. Mota, Heme oxygenase-1 and carbonmonoxide suppress the pathogenesis of experimental cerebral malaria, Nat. Med. 13 (2007) 703–710.
[42] F.H. Amante, A. Haque, A.C. Stanley, L. Rivera Fde, L.M. Randall, Y.A. Wilson, G. Yeo, C. Pieper, B.S. Crabb, T.F. de Koning-Ward, R.J. Lundie, M.F. Good, A. Pinzon-Charry, M.S. Pearson, M.G. Duke, D.P. McManus, A. Loukas, G.R. Hill, C.R. Engwerda, Immune-mediated mechanisms of parasite tissue sequestration during experimental cerebral malaria, J. Immunol. 185 (2010) 3632–3642.
[43] O. Prommano, U. Chaisri, G.D. Turner, P. Wilairatana, D.J. Ferguson, P. Viriyavejakul, N.J. White, E. Pongponratn, A quantitative ultrastructural study of the liver and the spleen in fatal falciparum malaria, Southeast Asian J. Trop. Med. Public Health 36 (2005) 1359–1370.
[44] J.C. Cuisinier-Raynal, F. Bire, M. Clerc, J. Bernard, J. Sarrouy, Human malaria: dysglobulinemia-hypocholesterolemia syndrome, Med. Trop. (Mars). 50 (1990) 91–95.
[45] J.F. Faucher, E. Ngou-Milama, M.A. Missinou, R. Ngomo, M. Kombila, P.G. Kremsner, The impact of malaria on common lipid parameters, Parasitol. Res. 88 (2002) 1040–1043.
[46] S. Mohanty, S.K. Mishra, B.S. Das, S.K. Satpathy, D. Mohanty, J.K. Patnaik, T.K. Bose, Altered plasma lipid pattern in falciparum malaria, Ann. Trop. Med. Parasitol. 86 (1992) 601–606.
[47] E.M. Kittl, G. Diridl, V. Lenhart, C. Neuwald, J. Tomasits, H. Pichler, K. Bauer, HDL cholesterol as a sensitive diagnostic parameter in malaria, Wien. Klin. Wochenschr. 104 (1992) 21–24.
[48] J.L. Baptista, T. Vervoort, P. Van der Stuyft, M. Wery, Changes in plasma lipid levels as a function of *Plasmodium falciparum* infection in Sao Tome, Parasite. 3 (1996) 335–340.
[49] B. Halliwell, Oxidative stress and neurodegeneration: where are we now? J. Neurochem. 97 (2006) 1634–1658.
[50] D. Bermpohl, A. Halle, D. Freyer, E. Dagand, J.S. Braun, I. Bechmann, Weber J.R. Schroder, Bacterial programmed cell death of cerebral endothelial cells involves dual death pathways, J. Clin. Investig. 115 (2005) 1607–1615.
[51] B.S. Das, N.K. Nanda, Evidence for erythrocyte lipid peroxidation in acute *falciparum* malaria, Trans. R. Soc. Trop. Med. Hyg. 93 (1999) 8–62.
[52] O. Erel, H. Vural, N. Aksoy, G. Aslan, M. Ulukanligil, Oxidative stress of platelets and thrombocytopenia in patients with *vivax* malaria, Clin. Biochem. 34 (2001) 341–344.
[53] M.J. Griffiths, F. Ndungu, K.L. Baird, D.P. Muller, K. Marsh, C.R. Newton, Oxidative stress and erythrocyte damage in Kenyan children with severe *Plasmodium falciparum* malaria, Br. J. Haematol. 113 (2001) 486–491.
[54] S.J. Heales, J.P. Bolanos, Impairment of brain mitochondrial function by reactive nitrogen species: the role of glutathione in dictating susceptibility, Neurochem. Int. 40 (2002) 469–474.
[55] U.J. Dumaswala, L. Zhuo, S. Mahajan, P.N. Nair, H.G. Shertzer, P. Dibello, D.W. Jacobsen, Glutathione protects chemokine-scavenging and antioxidative defense functions in human RBCs, Am. J. Phys. 208 (2001) 867–873.
[56] I. Gramaglia, P. Sobolewski, D. Meays, R. Contreras, J.P. Nolan, J.A. Frangos, M. Intaglietta, H.C. van der Heyde, Low nitric oxide bioavailability contributes to the genesis of experimental cerebral malaria, Nat. Med. 12 (2006) 1417–1422.
[57] R.P. Rother, L. Bell, P. Hillmen, M.T. Gladwin, The clinical sequelae of intravascular hemolysis and extracellular plasma hemoglobin: a novel mechanism of human disease, J. Am. Med. Assoc. 293 (2005) 1653–1662.
[58] K. Cosby, K.S. Partovi, J.H. Crawford, R.P. Patel, C.D. Reiter, S. Martyr, B.K. Yang, M.A. Waclawiw, G. Zalos, X. Xu, K.T. Huang, H. Shields, D.B. Kim-Shapiro, A.N. Schechter, R.O. Cannon 3rd, M.T. Gladwin, Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation, Nat. Med. 9 (2003) 1498–1505.
[59] M. Kakimoto, T. Inoguchi, T. Sonta, H.Y. Yu, M. Imamura, T. Etoh, T. Hashimoto, H. Nawata, Accumulation of 8-hydroxy-2′-deoxyguanosine and mitochondrial DNA deletion in kidney of diabetic rats, Diabetes. 51 (2002) 1588–1595.
[60] L.J. Kroese, P.G. Scheffe, 8-Hydroxy-2′-deoxyguanosine and cardiovascular disease: a systematic review, Curr. Atheroscler. Rep. 16 (2014) 452.
[61] A. Płachetka, B. Adamek, J.K. Strzelczyk, Ł. Krakowczyk, P. Migula, P. Nowak, A. Wiczkowski, 8-hydroxy-2′-deoxyguanosine in colorectal adenocarcinoma – is it a result of oxidative stress? Med. Sci. Monit. 19 (2013) 690–695.
[62] C. Punsawad, Y. Maneerat, U. Chaisri, K. Nantavisai, P. Viriyavejakul, Nuclear factor kappa B modulates apoptosis in the brain endothelial cells and intravascular leukocytes of fatal cerebral malaria, Malar. J. 12 (2013) 260.
[63] C. Punsawad, S. Krudsood, Y. Maneerat, U. Chaisri, N. Tangpukdee, E. Pongponratn, K. Nantavisai, R. Udomsangpetch, P. Viriyavejakul, Activation of nuclear factor kappa B in peripheral blood mononuclear cells from malaria patients, Malar. J. 11 (2012) 191.

[64] E.G. Novoselova, S.M. Lunin, T.V. Novoselova, M.O. Khrenov, O.V. Glushkova, N.V. Avkhacheva, V.G. Safronova, E.E. Fesenko, Naturally occurring antioxidant nutrients reduce inflammatory response in mice, Eur. J. Pharmacol. 615 (2009) (2009) 234–240.
[65] F.M. Sohet, A.M. Neyrinck, B.D. Pachikian, F.C. de Backer, L.B. Bindels, P. Niklowitz, T. Menke, P.D. Cani, N.M. Delzenne, Coenzyme Q_{10} supplementation lowers hepatic oxidative stress and inflammation associated with diet-induced obesity in mice, Biochem. Pharmacol. 78 (2009) 1391–1400.
[66] F.L. Crane, Biochemical functions of coenzyme Q_{10}, J. Am. Coll. Nutr. 20 (2001) 591–598.
[67] D.A. Groneberg, B. Kindermann, M. Althammer, M. Klapper, J. Vormann, G.P. Littarru, F. Döring, Coenzyme Q_{10} affects expression of genes involved in cell signalling, metabolism and transport in human CaCo-2 cells, Int. J. Biochem. Cell Biol. 37 (2005) 1208–1218.
[68] M.R. Safarinejad, S. Safarinejad, N. Shafiei, S. Safarinejad, Effects of the reduced form of coenzyme Q_{10} (ubiquinol) on semen parameters in men with idiopathic infertility: a double-blind, placebo controlled, randomized study, J. Urol. 188 (2012) 526–531.
[69] K. Toyama, S. Sugiyama, H. Oka, Y. Iwasaki, H. Sumida, T. Tanaka, S. Tayama, H. Jinnouchi, K. Matsui, H. Ogawa, Rosuvastatin combined with regular exercise preserves coenzyme Q_{10} levels associated with a significant increase in high-density lipoprotein cholesterol in patients with coronary artery disease, Atherosclerosis. 217 (2011) 158–164.
[70] S. Someya, J. Xu, K. Kondo, D. Ding, R.J. Salvi, T. Yamasoba, P.S. Rabinovitch, R. Weindruch, C. Leeuwenburgh, M. Tanokura, T.A. Prolla, Age-related hearing loss in C57BL/6J mice is mediated by Bak-dependent mitochondrial apoptosis, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 19432–19437.
[71] F.A. Kassa, K. Van Den Ham, A. Rainone, S. Fournier, E. Boilard, M. Olivier, Absence of apolipoprotein E protects mice from cerebral malaria, Sci. Rep. 6 (2016) 33615.
[72] Y. Cariaco, W.R. Lima, R. Sousa, L.A.C. Nascimento, M.P. Briceño, W.L. Fotoran, G. Wunderlich, J.L. Dos Santos, N.M. Silva, Ethanolic extract of the fungus *Trichoderma stromaticum* decreases inflammation and ameliorates experimental cerebral malaria in C57BL/6 mice, Sci. Rep. 8 (2018) 1547.
[73] S. Adams, H. Brown, G. Turner, Breaking down the blood–brain barrier: signaling a path to cerebral malaria? Trends Parasitol. 18 (2002) 360–366.
[74] J. Zhai, Y. Yacong Bo, Lu y, Liu C, Zhang L., Effects of Coenzyme Q_{10} on markers of inflammation: a systematic review and meta-analysis, PLoS One 12 (2017) 0170172.
[75] M. Sanoobar, S. Eghtesadi, A. Azimi, M. Khalili, B. Khodadadi, S. Jazayeri, M.R. Gohari, N. Aryaeian, Coenzyme Q_{10} supplementation ameliorates inflammatory markers in patients with multiple sclerosis: a double blind, placebo, controlled randomized clinical trial, Nutr. Neurosci. 18 (2015) 169–176.
[76] M. Prato, G. Giribaldi, Matrix metalloproteinase-9 and haemozoin: wedding rings for human host and plasmodium falciparum parasite in complicated malaria, J. Trop. Med. 2011 (2011) 628435.
[77] A. Szklarczyk, M. Stins, E.A. Milward, H. Ryu, C. Fitzsimmons, D. Sullivan, K. Conant, Glial activation and matrix metalloproteinase release in cerebral malaria, J. Neurovirol. 13 (2007) 2–10.
[78] P.E. Van den Steen, I. Van Aelst, S. Starckx, K. Maskos, G. Opdenakker, A. Pagenstecher, Matrix metalloproteinases, tissue inhibitors of MMPs and TACE in experimental cerebral malaria, Lab. Investig. 86 (2006) 873–888.
[79] M. Prato, G. Giribaldi, M. Polimeni, V. Gallo, P. Arese, Phagocytosis of hemozoin enhances matrix metalloproteinase-9 activity and TNF-alpha production in human monocytes: role of matrix metalloproteinases in the pathogenesis of falciparum malaria, J. Immunol. 175 (2005) 6436–6442.
[80] Y. Gursoy-Ozdemir, J. Qiu, N. Matsuoka, H. Bolay, D. Bermpohl, H. Jin, X. Wang, G.A. Rosenberg, E.H. Lo, M.A. Moskowitz, Cortical spreading depression activates and upregulates MMP- 9, J. Clin. Invest. 113 (2004) 1447–1455.
[81] V.W. Yong, C. Power, P. Forsyth, D.R. Edwards, Metalloproteinases in biology and pathology of the nervous system, Nat. Rev. Neurosci. 2 (2001) 502–511.
[82] M. Asahi, X. Wang, T. Mori, T. Sumii, J.C. Jung, M.A. Moskowitz, M.E. Fini, E.H. Lo, Effects of matrix metalloproteinase-9 gene knock-out on the proteolysis of blood–brain barrier and white matter components after cerebral ischemia, J. Neurosci. 21 (2001) 7724–7732.
[83] G.D. Turner, H. Morrison, M. Jones, T.M. Davis, S. Looareesuwan, I.D. Buley, K.C. Gatter, C.I. Newbold, S. Pukritayakamee, B. Nagachinta, N.J. White, A.R. Berendt, An immunohistochemical study of the pathology of fatal malaria. Evidence for widespread endothelial activation and a potential role for intercellular adhesion molecule-1 in cerebral sequestration, Am. J. Pathol. 145 (1994) 1057–1069.
[84] V.H. Tchinda, A.D. Tadem, E.A. Tako, G. Tene, J. Fogako, P. Nyonglema, G. Sama, A. Zhou, R.G. Leke, Severe malaria in Cameroonian children: correlation between plasma levels of three soluble inducible adhesion molecules and TNF-alpha, Acta Trop. 102 (2007) 20–28.
[85] Q. de Mast, E. Groot, P.J. Lenting, P.G. de Groot, M. McCall, R.W. Sauerwein, R. Fijnheer, A. van der Ven, Thrombocytopenia and release of activated von Willebrand factor during early *Plasmodium falciparum* malaria, J. Infect. Dis. 196 (2007) 622–628.
[86] M.J. Hollestelle, C. Donkor, E.A. Mantey, S.J. Chakravorty, A. Craig, A.O. Akoto, J. O'Donnell, J.A. van Mourik, J. Bunn, von Willebrand factor propeptide in malaria: evidence of acute endothelial cell activation, Br. J. Haematol. 133 (2006) 562–569.
[87] T.W. Yeo, D.A. Lampah, R. Gitawati, E. Tjitra, E. Kenangalem, K. Piera, R.N. Price, S.B. Duffull, D.S. Celermajer, N.M. Anstey, Angiopoietin-2 is associated with decreased endothelial nitric oxide and poor clinical outcome in severe falciparum malaria, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 17097–17102.
[88] F.E. Lovegrove, N. Tangpukdee, R.O. Opoka, E.I. Lafferty, N. Rajwans, M. Hawkes, S. Krudsood, S. Looareesuwan, C.C. John, W.C. Liles, C. Kevin, K.C. Kain, Serum angiopoietin-1 and -2 levels discriminate cerebral malaria from uncomplicated malaria and predict clinical outcome in African children, PLoS One 4 (2009) 4912.
[89] A. Kumar, H. Kaur, P. Devi, V. Mohan, Role of coenzyme Q_{10} (CoQ_{10}) in cardiac disease, hypertension and Meniere-like syndrome, Pharmacol. Ther. 124 (2009) 259–268.
[90] S. Pepe, S.F. Marasco, S.J. Haas, F.L. Sheeran, H. Krum, F.L. Rosenfeldt, Coenzyme Q_{10} in cardiovascular disease, Mitochondrion. 7 (2007) 54–67.
[91] G. Cairo, S. Recalcati, A. Mantovani, M. Locati, Iron trafficking and metabolism in macrophages: contribution to the polarized phenotype, Trends Immunol. 32 (2011) 241–247.
[92] R. Motterlini, R. Foresti, R. Bassi, C.J. Green, Curcumin, an antioxidant and anti-inflammatory agent, induces heme oxygenase-1 and protects endothelial cells against oxidative stress, Free Radic. Biol. Med. 28 (2000) 1303–1312.
[93] K.N. Couper, D.G. Blount, M.S. Wilson, J.C. Hafalla, Y. Belkaid, M. Kamanaka, R.A. Flavell, J.B. de Souza, E.M. Riley, IL-10 from CD4CD25Foxp3CD127 adaptive regulatory T cells modulates parasite clearance and pathology during malaria infection, PLoS Pathol. 4 (2008) 1000004.
[94] M.A. Itoe, J.L. Sampaio, G.G. Cabal, Host cell phosphatidylcholine is a key mediator of malaria parasite survival during liver stage infection, Cell Host Microbe 16 (2014) 778–786.
[95] S. Ghosh, A. Sengupta, S. Sharma, H.M. Sonawat, Metabolic fingerprints of serum, brain, and liver are distinct for mice with cerebral and noncerebral malaria: A1H NMR spectroscopy-based metabonomic study, J. Proteome Res. 11 (2012) 4992–5004.
[96] Z. Xu, J. Huo, X. Ding, M. Yang, L. Li, J. Dai, K. Hosoe, H. Kubo, M. Mori, K. Higuchi, J. Sawashita, Coenzyme Q_{10} improves lipid metabolism and ameliorates obesity by regulating CaMKII mediated PDE4 inhibition, Sci. Rep. 7 (2017) 8253.
[97] V.B. Antappanavar, S.G. Biradar, V. Patil, P.M. Biradar, S. Mithare, A.K. Sharma, A study of correlation between iron deficiency anaemia and serum lipid profile in Indian adults in BRIMS, Bidar, Int. J. Adv. Med. 1 (2014) 96–100.
[98] S.V. Avery, Molecular targets of oxidative stress, Biochem. J. 434 (2011) 201–210.
[99] G.M. Zanini, Y.C. Martins, P. Cabrales, J.A. Frangos, L.J. Carvalho, S-nitrosoglutathione prevents experimental cerebral malaria, J. NeuroImmune Pharmacol. 7 (2012) 477–487.
[100] K. Aoyama, M. Watabe, T. Nakaki, Regulation of neuronal glutathione synthesis, J. Pharmacol. Sci. 108 (2008) 227–238.
[101] N. Ballatori, S.M. Krance, S. Notenboom, S. Shi, K. Tieu, C.L. Hammond, Glutathione dysregulation and the etiology and progression of human diseases, Biol. Chem. 390 (2009) 191–214.
[102] R.M. Solano, M.J. Casarejos, J. Menendez-Cuervo, J.A. Rodriguez-Navarro, J.G. Yebenes, M.A. Mena, Glial dysfunction in parkin null mice: effects of aging, J. Neurosci. 28 (2008) 598–611.
[103] C.G. Fraga, M.K. Shigenaga, J.W. Park, P. Degan, B.N. Ames, Oxidative damage to DNA during aging: 8-hydroxy-2_-deoxyguanosine in rat organ DNA and urine, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 4533–4537.
[104] D.A. Merriwether, A.G. Clark, S.W. Ballinger, T.G. Schurr, H. Soodyall, T. Jenkins, S.T. Sherry, D.C. Wallace, The structure of human mitochondrial DNA variation, J. Mol. Evol. 33 (1991) 543–555.
[105] Y.C. Martins, G.M. Zanini, J.A. Frangos, L.J. Carvalho, Efficacy of different nitric oxide-based strategies in preventing experimental cerebral malaria by *Plasmodium berghei ANKA*, PLoS One 7 (2012) 32048.
[106] N.M. Anstey, J.B. Weinberg, M.Y. Hassanali, E.D. Mwaikambo, D. Manyenga, M.A. Misukonis, D.R. Arnelle, D. Hollis, M.I. McDonald, D.L. Granger, Nitric oxide in Tanzanian children with malaria: inverse relationship between malaria severity and nitric oxide production/nitric oxide synthase type 2 expression, J. Exp. Med. 184 (1996) 557–567.
[107] B.K. Lopansri, N.M. Anstey, J.B. Weinberg, G.J. Stoddard, M.R. Hobbs, M.C. Levesque, E.D. Mwaikambo, D.L. Granger, Low plasma arginine concentrations in children with cerebral malaria and decreased nitric oxide production, Lancet. 361 (2003) 676–678.
[108] C.R. Morris, G.J. Kato, M. Poljakovic, X. Wang, W.C. Blackwelder, V. Sachdev, S.L. Hazen, E.P. Vichinsky, S.M. Morris Jr., M.T. Gladwin, Dysregulated arginine metabolism, hemolysis-associated pulmonary hypertension, and mortality in sickle cell disease, J. Am. Med. Assoc. 294 (2005) 81–90.
[109] W. Li, Y. Zhao, X. Xu, W. Ma, P. Gao, Y. Wang, K. Liang, R. Li, Rebamipide suppresses TNF-a mediated inflammation in vitro and attenuates the severity of dermatitis in mice, FEBS J. 282 (2015) 2317–2326.
[110] J. Zhu, G. Krishnegowda, D.C. Gowda, Induction of proinflammatory responses in macrophages by the glycosylphosphatidylinositols of Plasmodium falciparum: the requirement of extracellular signal-regulated kinase, p38, c-Jun N-terminal kinase and NF-kappaB pathways for the expression of proinflammatory cytokines and nitric oxide, J. Biol. Chem. 280 (2005) 8617–8627.
[111] M. Jaramillo, D.C. Gowda, D. Radzioch, M. Olivier, Haemozoin increases IFNgamma- inducible macrophage nitric oxide generation through extracellular signal-regulated kinase- and NF-kappa B-dependent pathways, J. Immunol. 171 (2003) 4243–4253.
[112] S.D. Tachado, P. Gerold, M.J. McConville, T. Baldwin, D. Quilici, R.T. Schwarz, L. Schofield, Glycosylphosphatidylinositol toxin of Plasmodium induces nitric oxide synthase expression in macrophages and vascular endothelial cells by a protein tyrosine kinase-dependent and protein kinase C-dependent signaling pathway, J. Immunol. 156 (1996) 1897–1907.
[113] Y.P. Zhang, A. Eber, Y. Yuan, Z. Yang, Y. Rodriguez, R.C. Levitt, P. Takacs,

K.A. Candiotti, Prophylactic and antinociceptive effects of coenzyme Q10 on diabetic neuropathic pain in a mouse model of type 1 diabetes, Anesthesiology. 118 (2013) 945–954.

[114] C. Schmelzer, H. Kubo, M. Mori, J. Sawashita, M. Kitano, K. Hosoe, I. Boomgaarden, F. Döring, K. Higuchi, Supplementation with the reduced form of coenzyme Q_{10} decelerates phenotypic characteristics of senescence and induces a PPAR-alpha gene expression signature in SAMP1 mice, Mol. Nutr. Food Res. 54 (2009) 805–815.

[115] C. Schmelzer, I. Lindner, G. Rimbach, P. Niklowitz, T. Menke, F. Döring, Functions of coenzyme Q_{10} in inflammation and gene expression, Biofactors. 32 (2008) 179–183.

[116] C. Schmelzer, G. Lorenz, G. Rimbach, F. Doring, *In vitro* effects of the reduced form of coenzyme Q_{10} on secretion levels of TNF-alpha and chemokines in response to LPS in the human monocytic cell line THP-1, J. Clin. Biochem. Nutr. 44 (2009) 62–66.

[117] H. Nohl, N. Rohr-Udilova, L. Gille, W. Bieberschulte, D. Jurek, B. Marian, R. Schulte-Herman, Suppression of tumour-promoting factors in fat- induced colon carcinogenesis by the antioxidants caroverine and ubiquinone, Anticancer Res. 25 (2005) 2793–2800.