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Mefloquine-curcumin combinations improve host mitochondrial respiration and decrease mitotoxic effects of mefloquine in *Plasmodium berghei*-infected mice

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ABSTRACT

Plasmodium infection is a health challenge. Although, antiplasmodial drugs kill the parasites, information on the effects of infection and drugs on the expression of some genes is limited.

Malaria was induced in two different studies using NK65 (chloroquine-susceptible, study 1), and ANKA (chloroquine-resistant, study 2) strains of *Plasmodium berghei* in 30 male Swiss mice (n = 5) in each study. Mice orally received 10 mL/kg distilled water, (infected control), Mefloquine (MF) (10 mg/kg), MF and Curcumin (CM) (25 mg/kg), MF and CM (50 mg/kg), CM (25 mg/kg) and CM (50 mg/kg). Five mice (un-infected) were used as the control. After treatment, total Ribonucleic acid (RNA) was isolated from liver and erythrocytes while Deoxyribonucleic acid (DNA)-free RNA were converted to cDNA. Polymerase Chain Reaction (PCR) amplification was performed and relative expressions of *FIKK12, AQP3, P38 MAPK, NADH* oxidoreductase, and cytochrome oxidase expressions were determined. Markers of glycolysis, toxicity and antioxidants were determined using ELISA assays. While the expression of *FIKK12* was blunted by MF in the susceptible study, co-treatment with curcumin (25 mg/kg) yielded the same results in the chloroquine-resistant study. Similar results were obtained on *AQP3* in both studies. Curcumin decreased *P38 MAPK* in both studies. *Plasmodium* infection decreased *NADH* oxidoreductase and cytochrome oxidase but mefloquine-curcumin restored the expression of these genes. While glycolysis and toxicity were inhibited, antioxidant systems improved in the treated groups. Curcumin is needed for effective therapeutic efficacy and prevention of toxicity. *Plasmodium* infection and treatment modulate the expressions of some genes in the host. Curcumin combination with mefloquine modulates the expression of some genes in the host.

1. Background

There are quite a number of parasites that infect humans but, according to the World Health Organisation report, infection by the *Plasmodium falciparum* accounts for the highest fatal incidence with the highest recorded deaths (World Health Organization, 2018). During the blood meal by the host (female anopheles mosquito), *Plasmodium*-containing saliva is injected into the host, resulting in infection. This is the beginning of the pre-erythrocytic stage, where injected sporozoites are transported into the liver. The parasite is able to evade the host preventive machinery through some proteins as reported by Angel and Linas (2021). Of all the host membrane proteins hijacked by the parasite for its survival, aquaporin3 (AQP3) is one of the most critical, being a water and glycerol channel. It contains six transmembrane helices and it is expressed throughout the body as reported by Bruguera and Herrera (2007). It is hijacked for the maturation of the parasite after the invasion of the host liver tissue (Posfai et al. 2018, 2020) Phosphorylation of the host plasma membrane cytoskeletal proteins such as ankyrin, spectrin, band 3 and protein 4.1 meshwork has been reported (Nunes et al., 2010). Many cell types such as macrophages, dendritic cells, platelets, and endothelial cells, express CD36 according to Chaurasiya et al. (2021). CD36-mediated phagocytosis of pathogens and

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pathogenic molecules activates Src/Syk kinases, leading to the activation of ERK, p38 and Jun members of the MAPK signaling pathways and NF-kB (Stewart et al., 2010; Cabrera et al., 2014). *P38 MAPK* is expressed throughout the body and previous studies have shown that CD36-induced MAPK signaling contributes to the production of TNF-α in mouse malaria infection and modulates parasite glycosylphosphatidyl inositol-induced cytokine responses in mouse macrophages and human blood dendritic cells (Patel et al., 2007; Erdman et al., 2009; Gowda et al., 2013). Glycolysis controls cellular energy and bio-synthetic processes in the cell. Antiglycolytic agents are currently under investigation in the treatment of microbial diseases, including malaria. Disruption of host glycolysis is highly valued as a therapeutic strategy since this process is tolerated by humans and other mammals but not parasites (Jezewski et al., 2021). Malaria parasites have complex mitochondrial metabolism connected to oxidative phosphorylation. Therefore, the high metabolic demand turns glycolysis into an essential energy generating process (Verlinde et al., 2001; Subramanian et al., 2015; Shah-Simpson et al., 2017). A previous study has shown that these parasites are aerobic glycolytic organisms with a modified Tricarboxylic acid (TCA) mainly fueled by glutamine and glutamate and low oxidative phosphorylation (Salcedo-Sora et al., 2014). In essence, survival of parasites in the host will depend largely on the regulation of host glycolysis. Mefloquine is an established drug for malaria treatment. It has been suggested previously that the antiplasmodial mechanism of action of mefloquine is via eryptosis (Bissinger et al., 2015). The effects of mefloquine on the expression of *FIKK, aquaporin-3*, *MAPK* and oxidative phosphorylation via the expression of some electron transport proteins and the modulatory roles of curcumin in these processes have not been reported. Parasitic infection causes imbalance in the redox system of the host leading to increase in the production of reactive oxygen species. This causes oxidative stress that leads to systemic failure and organ damage. In order to overcome this challenge, supplementation of treatment with antioxidants is a critical way to fight free radicals generated by the infection (Percário et al., 2012). In most cases, host defense system is not sufficient during malaria infection, there is therefore, a need for antioxidant supplementation (Ekeh et al., 2019). Recent studies have shown that supplementation of antimalarial drugs with antioxidants strengthens the endogenous antioxidant system against oxidative stress or acting indirectly towards parasite destruction (Gomes et al., 2022). Antimalarial drugs have been supplemented with antioxidants such as N-acetyl cysteine, fenozyme, L-glutathione and melatonin for antiplasmodial efficacy (Gomes et al., 2022). Zhao et al. (2019) opined that fenozyme combination with an antimalarial drug may provide a new insight into malaria treatment strategy. Curcumin, (diferuloylmethane) is a naturally occurring phenolic compound and it has been proven to have antimalarial potentials against *Plasmodium* species (Nandakumar et al., 2006; Cui and Miao, 2007). We hypothesized therefore, that mefloquine's multiple molecular mechanistic role as an antiplasmodial agent involves serine/threonine kinase inhibition and aquaporin-3 gene silencing (Cooper and Brown, 2008). This study will provide information on the role of curcumin on host mitochondrial respiration, glycolysis, signaling through the MAPK pathway, and antioxidant status in both susceptible and resistant malaria using *Plasmodium berghei* as the causative agent (Febbraio et al. 2001).

In this study, we have analyzed the molecular mechanistic roles of mefloquine on the ablation of *FIKK12* in erythrocytes of infected mice, the down-regulation of aquaporin-3, *P38 MAPK*, some glycolytic enzymes and how supplementation of mefloquine with curcumin normalizes this situation and exacerbates the expression of some mitochondrial electron transport proteins (Isah and Ibrahim, 2014). We showed that curcumin significantly decreases phosphorylation of infected erythrocytes membrane skeleton proteins and decreases the recruitment of host aquaporin-3 and expression of *MAPK* for parasite advantage (McGill and Jaeschke, 2021). We also showed that since the parasite depends mainly on glycolysis for energy generation and less on oxidative phosphorylation, complementing mefloquine treatment with curcumin decreased the activity of host glycolytic enzymes and up-regulated the activities of mitochondrial electron transport proteins via an increase in their gene expression, linking these effects to a decrease in oxidative stress and improved antioxidant processes in the host (Percário et al. 2012).

2. Methods

2.1. Experimental animals

Forty male Swiss mice (18 ± 2 g) were obtained from the Animal House Section of the Malaria Research at Institute of Advanced Medical Research and Training. Thirty-five of these were infected with erythrocytes obtained from a donor mouse infected with chloroquine susceptible (NK 65) *Plasmodium berghei*. Parasitemia was confirmed after 72 h and the mice were grouped as follows: infected control (treated with 10 mL/kg vehicle), mefloquine treated (10 mg/kg), mefloquine treatment supplemented with curcumin (25 and 50 mg/kg) and curcumin treatment only (25 and 50 mg/kg). Supplementation with curcumin took place after mefloquine had earlier been administered. Another set of five mice that were not infected were treated with vehicle (10 mL/kg). Mice were treated orally and once daily for seven days. Slides were prepared at two days interval for microscopy. In another experiment, this same grouping and treatment of animals was adopted but only the mice were infected with resistant strain (ANKA) of *Plasmodium berghei*. Slides were prepared at two days interval.

2.2. Sample preparation

2.2.1. Serum preparation

After the treatment, mice were sacrificed through the injection of sodium pentobarbital (80 mg/kg). Blood was collected into plain sample bottles and allowed to clot. These were spun in the refrigerated centrifuge (Sigma 300-K, Germany) at 3000 rpm for 5 min and the serum aspirated into other sample bottles, labeled, kept in the fridge and used within two days. One drop of blood from the animals in each group was preserved in 70 μL of trizol for RNA isolation. Similarly, small quantity of liver tissue were fixed in trizol for RNA isolation.

2.2.2. Preparation of post-mitochondrial fraction

Liver samples were excised and rinsed in mitochondrial isolation buffer (210 mM Mannitol, 70 mM Sucrose, 5 mM 4-(2-hydroxymethyl) ethanesulfonic acid basified with potassium hydroxide (Hepes-KOH, pH 7.4) and 1 mM EGTA). The liver samples were then chopped using a scissors and a 10% suspension was homogenized using porter Elvejem homogenizer at one stroke per 2 s until the liver samples were completely homogenized. The homogenates were spun in the cold centrifuge at 13,000 rpm. The cold supernatant was aspirated into clean sample bottles and kept in the refrigerator until used.

2.2.3. Liver and spleen samples preparation for tissue histology

The tissues were observed and cut into small pieces of not more than 4 mm thick into pre-labeled cassettes. These were further immersed in 10% formal saline for 24 h to fix. Tissue processing was done automatically using automatic tissue processor (Leica TP 1020). The tissues were allowed to pass through various reagents including; stations 1 & 2 containing 10% formal saline, station 3 to station 7; alcohol (70%, 80%, 90%, 95%, absolute 1 & absolute 11) for dehydration. The tissues continued to pass through station 8 and station 9 containing two changes of xylene for the purpose of clearing and finally transferred into three wax baths for infiltration/impregnation. The machine has been programmed to run for 12 h, tissues stayed in each station for 1 h.

Each processed tissue was given paraffin wax as a solid support medium and this was done using a semi-automatic tissue embedding center. The molten paraffin wax was dispensed into a metal mold and the tissue was buried and oriented in it; a pre labeled cassette was placed

on this and then transferred to a cold plate to solidify. The tissue block formed was separated from the mold. The blocks were trimmed to expose the tissue surface using a rotary microtome at 6 μm. The surfaces were allowed to cool on ice before sectioning. The tissues were sectioned at 4 μm. The sections were thereafter, floated on water bath (Raymond lamb) set at 55 °C and these were picked using clean slides. The slides were labeled and dried on a hotplate (Raymond lamb) set at 60 °C for 1 h. The Haematoxylin and Eosin staining technique was used (Avwioro, 2010).

2.2.4. Total RNA isolation

Total RNA was isolated from whole liver and blood samples following the method described by Omotuyi et al. (2018). Briefly, liver tissues were homogenized in cold (4 °C) TRI reagent (Zymo Research, USA, Cat: R2050-1-50, Lot: ZRC186885). Total RNA was partitioned in chloroform (BDH Analytical Chemicals, Poole, England Cat: 10076-6B) following centrifugation at 15,000 rpm/15 min (Abbott Laboratories, Model: 3531, Lake Bluff, Illinois, United States). RNA from the clear supernatant was precipitated using an equal volume of isopropanol (Burgoyne Urbidges & Co, India, Cat: 67-63-0). The RNA pellet was rinsed twice in 70% ethanol (70 ml absolute ethanol (BDH Analytical Chemicals, Poole, England Cat: 10107-7Y) in 30 ml of nuclease-free water (Inqaba Biotec, West Africa, Lot no: 0596C320, code: E476-500 ML)). The pellets were air-dried for 5 min and dissolved in RNA buffer (1 mM sodium citrate, pH 6.4)

2.2.5. cDNA conversion

Prior to cDNA conversion, total RNA quantity (concentration (μg/ml) = 40 * A_{260}) and quality (≥1.8) was assessed using the ratio of A_{260}/A_{280} (A = absorbance) read using a spectrophotometer (Jen-way UV-VIS spectrophotometer model 6305, UK). DNA contamination was removed from RNA following DNAse I treatment (NEB, Cat: M0303S) as specified by the manufacturer. A 2 μl solution containing 100 ng DNA-free RNA was converted to cDNA using M-MuLV Reverse transcriptase Kit (NEB, Cat: M0253S) in 20 μl final volume (2 μl, N^9 random primer mix; 2 μl, 10X M-MuLV buffer; 1 μl, M-MuLV RT (200 U/μl); 2 μl, 10 mM dNTP; 0.2 μl, RNase Inhibitor (40 U/μl) and 10.8 μl nuclease-free water). The reaction proceeded at room temperature O/N. Inactivation of M-MuLV Reverse transcriptase was performed at 65 °C/20 min.

2.2.6. PCR amplification and agarose gel electrophoresis

PCR amplification for the determination of genes whose primers (Primer3 software) are listed below (Table 1) were done using the following protocol: PCR amplification was performed in a total of 25 μl volume reaction mixture containing 2 μl cDNA (10 ng), 2 μl primer (100 pmol) 12.5 μl Ready Mix Taq PCR master mix (One Taq Quick-Load 2x, master mix, NEB, Cat: M0486S) and 8.5 μl nuclease-free water. Initial denaturation at 95 °C for 5 min was followed by 20 cycles of amplification (denaturation at 95 °C for 30 s, annealing for 30 s and extension at 72 °C for 60 s and ending with final extension at 72 °C for 10 min. In all experiments, negative controls were included where reaction mixture has no cDNA. The amplicons were resolved on 1.5% agarose gel (Cleaver Scientific Limited: Lot: 14170811) in Tris (RGT reagent, china, Lot: 20170605)-Borate (JHD chemicals, China, Lot 20141117)-EDTA buffer (pH 8.4).

2.3. Biochemical assays

2.3.1. Amplicon image processing and quantitation

In-gel amplicon bands images captured on camera were processed on Keynote platform as previously reported and quantified using image-J software.

2.3.2. Determination of lactate dehydrogenase, aldolase and glutamate dehydrogenase

Activities of serum lactate dehydrogenase, aldolase and glutamate dehydrogenase were determined by using ELISA assay kits obtained from Elabscience (USA).

2.3.3. Assay of immunological changes in the experimental animals

The levels of immunoglobulin M (IgM) and immunoglobulin G (IgG) were determined in the serum of the experimental animals according to the kits manufacturers' protocols (Fortress Diagnostics, Antrim, U.K).

2.3.4. Determination of post-mitochondrial fraction protein content

The method described by Lowry et al. (1951) was employed to determine total mitochondrial protein content. Post-mitochondrial fraction (10 μl) was added to freshly prepared alkaline copper solution (a mixture of 2% sodium trioxocarbonate (iv) (Na_2CO_3) in 0.1 M sodium hydroxide (NaOH), 2% Na–K-Tartrate, and 1% copper (II) tetraoxosulfate (vi) pentahydrate ($CuSO_4.5H_2O$) in 100:1:1 proportion, respectively). The assay volume in each test tube was uniformly made up with distilled water and the test tube contents were incubated at room temperature (30 °C) for 10 min. Folin-Ciocalteu reagent (0.3 mL of a five-fold dilution of a 2N stock) was added and the mixture vortexed. The mixture was left for another 30 min and the absorbance read at 750 nm using a spectrophotometer. Post-mitochondrial protein content was estimated from a protein standard curve using bovine serum albumin.

2.3.5. Determination of catalase activity

Catalase activity was determined in the post-mitochondrial fraction according to the method of Claiborne (1985).

Hydrogen peroxide (2.95 ml of 19 mM solution) was pipetted into a 1 cm quartz cuvette and 50 μl of sample added. The mixture was rapidly inverted to mix and placed in a spectrophotometer. Change in absorbance was read at 240 nm every minute for 5 min.

Calculation

$$\text{Catalase activity} = \frac{\Delta A_{240}/\text{min} \times \text{reaction volume} \times \text{dilution factor}}{0.0436 \times \text{sample volume} \times \text{mg protein/ml}}$$

$$= \mu\text{mole } H_2O_2/\text{min/mg protein}$$

2.3.6. Determination of superoxide dismutase activity

The activity of SOD was determined in the post-mitochondrial fraction by the method of Misra and Fridovich (1972). A 50 μl sample was added to 2.5 ml of 0.05M carbonate buffer (pH 10.2) and 0.3 ml of epinephrine in a cuvette, mixed by inversion and change in absorbance monitored every 30 s for 2.5 min at 480 nm. The reference cuvette was the same as for the samples with water replacing the samples.

Calculation

Table 1
List of Primer sequences for PCR assay.

S/N	Gene	Forward Sequence (5′-3′)	Reverse Sequence (5′-3′)
1	NADH Reductase	AGA CAT CAG GTT TAC GGG GC	CTC TCG GTC CTT ACA GGT GC
2	Aquaporin-3	TGC CTT GCG CTA GCT ACT TT	GAA GCA TCT CCC CAC AAC GA
3	FIKK12	GAA CTG TGG CCA TAT GTG GAA	AGT TGA CGG ATA GCA ACT CAA
4	Cytochrome C oxidase	CCT TGG ACG GCG GAA TG	TTC ACA ACA CTC CCA TGT GCT
5	p38-MAPK	CCAAGCCATGAGGCAAGAAA	GGCTGCTGTGATCCTCTTATC

$$\text{Percentage inhibition} = \frac{100 - (100 \times \text{Increase in absorbance perminforsample})}{\text{Increase in absorbance per min for blank}}$$

1 unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the autooxidation of epinephrine.

2.3.7. Estimation of reduced glutathione

The method of Beutler et al. (1963) was followed in estimating the level of reduced glutathione (GSH) in the post-mitochondrial fraction. A 0.4 ml sample was added to 0.4 ml of precipitating solution which was vortexed and centrifuged at 4000 rpm for 5 min. Thereafter, 0.5 ml supernatant was added to 1.5 ml of Ellman's reagent. The absorbance of the reaction mixture was read at 412 nm against a reagent blank. Reduced glutathione concentration in the sample was estimated from a standard curve.

2.3.8. Assay for glutathione peroxidase activity

Glutathione peroxidase (GPx) activity was measured in the post-mitochondrial fraction according to the procedure of Rotruck et al. (1973) with some modifications. To 0.5 ml phosphate buffer in a test tube was added 0.1 ml of NaN_3, 0.2 ml of GSH, 0.1 ml of H_2O_2 and 0.5 ml of sample. The reaction mixture was incubated for 3 min at 37 °C after which 0.5 ml of TCA was added and the final mixture centrifuged at 3000 rpm for 5 min. To 1 ml of the supernatants, 2 ml K_2HPO_4 and 1 ml DTNB were added and the absorbance read against a reagent blank of 1 ml distilled water, 2 ml K_2HPO_4 and 1 ml DTNB at 412 nm.

Calculation

$$\text{GSH consumed} = \frac{\text{initial GSH amount } (129.39\ \mu g) - \text{GSH remaining } (\mu g/ml \times 4\ ml)}{\text{GPX activity} = \text{GSH consumed}/\text{mg protein}}$$

$$= \mu g\ \text{GSH}/\text{mg protein}$$

2.3.9. Estimation of glutathione S-transferase activity

Glutathione S-transferase activity was determined in the post-mitochondrial fraction according to Habig et al. (1974). The medium for the estimation was prepared as follows: the cuvette containing the sample (30 μL) also contained 150 μL of 20 mM CDNB, 30 μL of 0.1 M reduced glutathione and 2.79 mL of 0.1 M phosphate buffer (pH 6.5). The cuvette for the blank only contained CDNB, reduced glutathione and phosphate buffer of the same volume and concentrations. The reaction for both blank and sample were allowed to run for 3 min with readings taken every 60 s against the blank at 340 nm.

Calculations.

The extinction coefficient of CDNB at 340 nm = 9.6 $mM^{-1}cm^{-1}$

$$\text{GSH S-transferase activity} = \frac{\Delta A_{340}/\text{min} \times \text{reaction volume} \times \text{dilution factor}}{9.6 \times \text{sample volume} \times \text{mg protein/ml}}$$

$$= \mu\text{mole}/\text{min}/\text{mg protein}$$

2.3.10. Biochemical assays for AST, ALT and GGT

Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyl transferase (GGT) activities were determined using ELISA assay kits (DIALAB Produktion und Vertrieb von chemisch, Wiener Neudorf, Austria), and the assay was carried out according to the manufacturer's procedures.

2.3.11. Assessment of lesions in liver and spleen tissues

The embedded tissue was de-waxed in Xylene for 15 min after which it was taken through a decreasing concentration of ethanol from absolute (100%) to 95% and lastly 70% alcohol. The section was taken through rinsing in water, stained in Harris haematoxylin for 5 min, rinsed in water again and differentiated briefly in 1% acid alcohol and rinsed in water again. The section was counter-stained with 1% aqueous Eosin for 2 min and rinsed in water. It was dehydrated in ascending grades of alcohol, cleared in xylene and mounted in DPX. The nucleus was stained blue while the cytoplasm was stained pink.

2.3.12. Statistical analysis

The experiments were conducted in triplicate and repeated twice. The means were calculated using at least three replicates of data. Error bars represent standard deviation (SD) for three independent experiments. Data were expressed as mean ± standard deviation of triplicate readings. Ungrouped data from this study were analyzed using One-way ANOVA followed by Tukey's post hoc using GraphPad prism 7.0. The level of significance was set at $P < 0.05$.

3. Results

3.1. Mefloquine blunts FIKK12 and aquaporin-3 expressions and combination with curcumin inhibits P38 MAPK expression in P. berghei-infected mice

To understand other molecular mechanisms of mefloquine as a potent antiplasmodial agent, we investigated the effect of this drug on the expression of FIKK kinase that is translocated from the apicomplexan *Plasmodium* to the host erythrocytes. When mefloquine was used to treat infected mice in the susceptible study, this drug served as a FIKK kinase inhibitor as it blunted the expression of the protein ($P < 0.0001$) compared with the infected control. This effect was not dose dependent as there was no significant difference between the two (25 and 50 mg/kg) doses. It was discovered that combination of curcumin at 25 mg/kg with mefloquine significantly decreased FIKK 12 expression relative to mefloquine only (Fig. 1D). In the resistant study, a similar result was obtained as the inhibition of FIKK 12 protein was least observed in the groups treated with curcumin only. However, curcumin dose at 25 mg/kg was more effective than 50 mg/kg (Fig. 1A).

The extent of expression of aquaporin-3 (AQP3) in mice infected with *Plasmodium berghei* without treatment and when there was intervention with mefloquine and supplementation with curcumin was investigated. AQP3 is found in the Kupffer cells localized within the lumen of liver sinusoids. Mefloquine was observed to decrease the expression of *AQP3* relative to the infected control. A strain-specific activity of curcumin modulation was observed since supplementation with 50 mg/kg blunts the expression of *AQP3* in the chloroquine-susceptible study (Fig. 1E) compared with the resistant study (Fig. 1B). Moreover, supplementation with 50 mg/kg curcumin with mefloquine caused a complete silencing of the *AQP3* gene as observed in the normal control. Furthermore, expression of the *AQP3* gene was decreased significantly ($P < 0.0001$) by the treatment of infected mice with 25 mg/kg curcumin only compared with the 50 mg/kg dose. The therapeutic pattern of mefloquine and curcumin supplementation differ in the resistant study compared with the susceptible one. Although, mefloquine decreased *AQP3* expression in the resistant study compared with the infected control ($P < 0.0001$), curcumin supplementation with

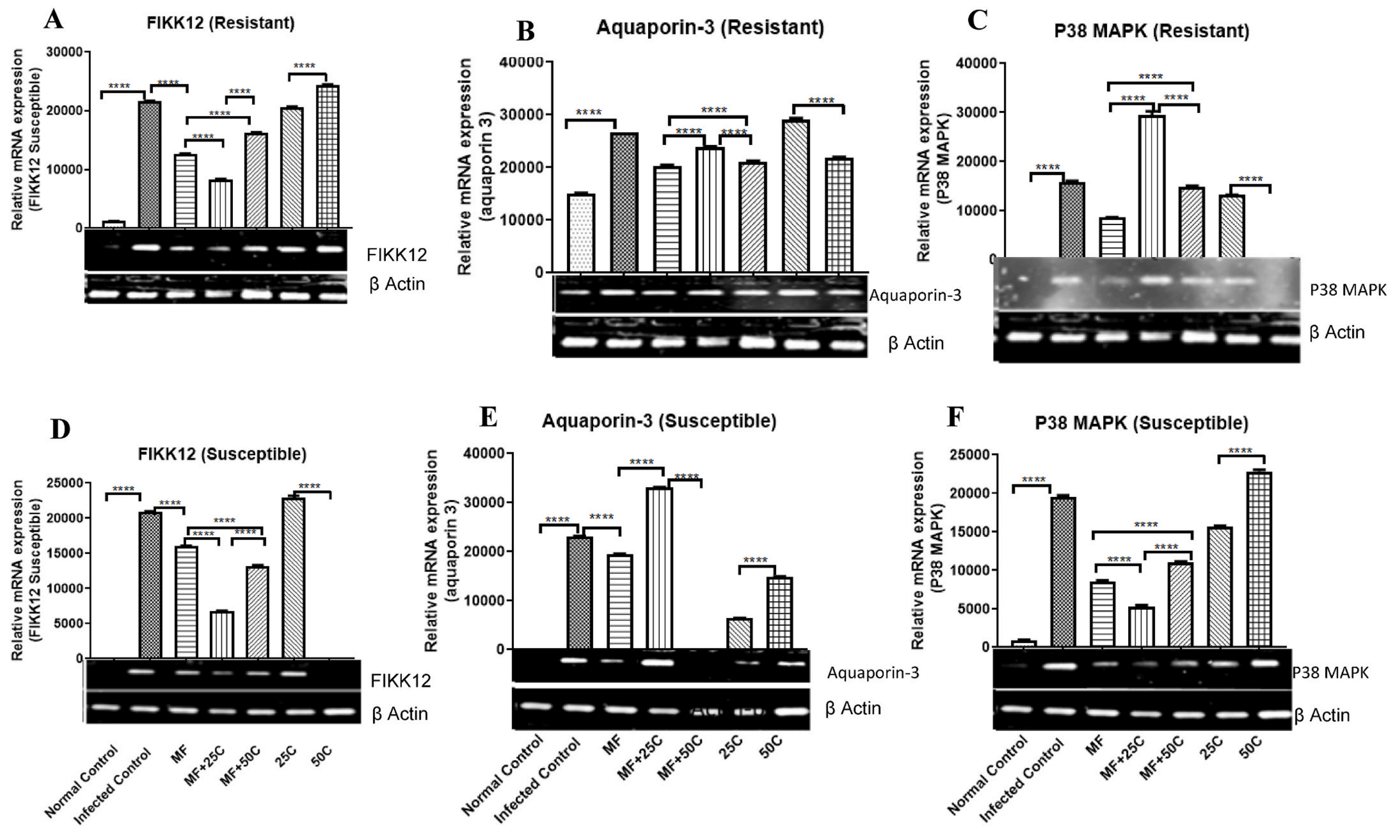

Fig. 1. The effects of mefloquine and curcumin combinations on the translocation of FIKK12, expressions of genes for aquaporin-3 and P38 MAPK in mice infected with resistant (A, B, C) and susceptible (D, E, F) strains of *P. berghei,* respectively. MF = Mefloquine; MF+25C = Mefloquine combined with 25 mg/kg curcumin, MF+50C = Mefloquine combined with 50 mg/kg curcumin; 25C = 25 mg/kg curcumin only; 50C = 50 mg/kg curcumin only. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; **** = $P < 0.0001$.

mefloquine at either dose did not decrease this effect. Further to this, supplementation of mefloquine treatment with curcumin, using the two doses, further increased *AQP3* expression ($P < 0.0001$) when compared with the drug only (Fig. 1B). The effect of treatment of mouse malaria with curcumin in both studies did not vary linearly with the dose.

The phosphorylation of P38 MAPK protein increase in the liver especially after toxin-induced liver injury. As observed in the normal control mice that were not infected, there was a complete ablation of this process especially in the susceptible study. Thereafter, there was a gradual decrease in the expression of MAPK; in other words, mefloquine only suppresses MAPK signaling in the treated infected mice and further decrease was observed when treatment with mefloquine was supplemented with curcumin ($P < 0.0001$) in the susceptible study. Curcumin only (either with 25 or 50 mg/kg dose) did not reduce the MAPK signaling effects (Fig. 1F). In the resistant study, an increase in curcumin dose is inversely proportional to *MAPK* expression and probably, a reflection of decrease in parasite load over time and the potency of mefloquine in the silencing of *MAPK* expression appeared not to be dose dependent (Fig. 1C).

3.2. Host mitochondrial electron transport system is affected by parasite infection but modulated by mefloquine treatment and curcumin supplementation

Although, the ultimate goal of the administration of an antimalarial drug to the host is parasite death, the molecular mechanism surrounding this event and the bio-energetic results in the host remains elusive. NADH oxidoreductase is protein enzyme in the inner mitochondrial membrane denoted as complex I in the electron transport chain. Untreated *Plasmodium* infection caused significant decrease in *NADH* reductase expression in mice while there was a marked expression in mice treated with mefloquine and further improvement when this treatment was supplemented with curcumin (maximally at 25 mg/kg) in the susceptible study (Fig. 2C).Similar to the susceptible study, curcumin supplementation up-regulated ($P < 0.0001$) *NADH* oxidoreductase gene expression in the resistant study (Fig. 2A). The evaluation of the expression of this gene in the two studies show that the relative expression of this gene is higher in the susceptible than the resistant study.

Cytochrome oxidase is another critical enzyme in the inner mitochondrial membrane denoted as complex IV in the electron transport chain. The extent of expression of cytochrome-c oxidase in mice infected with *P. berghei* that were not treated and those that were treated (chloroquine-susceptible and chloroquine-resistant strains) are presented in Fig. 2D (susceptible) and 2B (resistant).

This gene was the least expressed in the infected control in both studies. Treatment of mouse malaria with mefloquine up-regulated the expression of this gene in both studies. Supplementing mefloquine treatment of mouse malaria up-regulated the expression of this gene in both studies as well while maximum expression was dose dependent in the resistant study (Fig. 2B). It was also observed that supplementation of mefloquine treatment with both doses of curcumin did not upregulate the expression of the gene in the susceptible study (Fig. 2D) as it occurred in the resistant study. It is interesting to note that treatment of mouse malaria only using curcumin up-regulated ($P < 0.0001$) the expression of this gene maximally at 25 mg/kg (Fig. 2D and B).

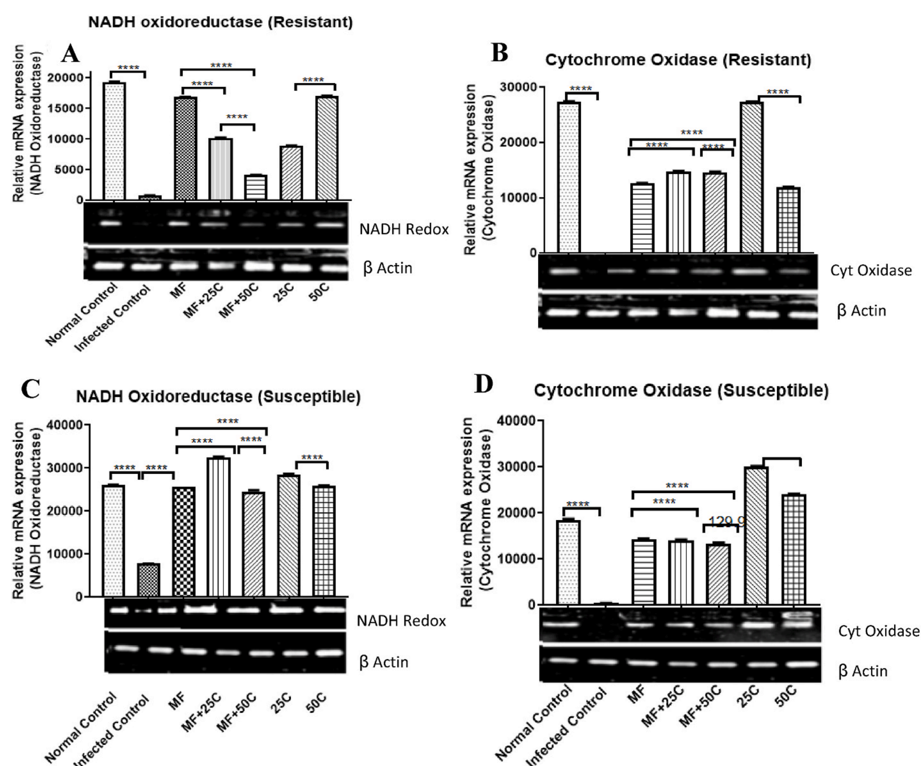

Fig. 2. The effect of mefloquine and curcumin combinations on the expressions of genes for mitochondrial complexes I (NADH oxidoreductase) and IV 9cytochrome oxidase in mice infected with resistant (A, B) and susceptible (C, D) strains of *P. berghei,* respectively. MF = Mefloquine; MF+25C = Mefloquine combined with 25 mg/kg curcumin, MF+50C = Mefloquine combined with 50 mg/kg curcumin; 25C = 25 mg/kg curcumin only; 50C = 50 mg/kg curcumin only. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; **** = $P < 0.0001$.

3.3. *Anti-glycolytic influence of mefloquine and the regulatory role of curcumin*

The anti-glycolytic effects of mefloquine and the modulatory roles of curcumin in this process was monitored via the determination of the activity of lactate dehydrogenase, aldolase and glutamate dehydrogenase as a key regulator of the entry of α-ketoglutarate to the TCA cycle with concomitant conversion of NADP to NADPH for other biosynthetic purposes.

It was observed that *Plasmodium* infection increased the activity of LDH in the infected control relative to the un-infected mice ($P < 0.0001$) and this effect was mitigated by mefloquine treatment ($P < 0.001$) and further by curcumin at 25 mg/kg. Curcumin treatment only did not have effect in decreasing the activity of this enzyme under the same condition in the susceptible study (Fig. 3A). In the resistant study, the ANKA strain similarly increased the activity of the serum LDH ($P < 0.001$) relative to the normal control but this effect was significantly reduced by mefloquine only and its supplementation with curcumin by either of the two doses ($P < 0.01$, in each case). The effect of curcumin treatment only did not vary linearly with the dose administered (Fig. 3B).

Overall, extreme deviations in the activities of these enzymes were not observed either as a result of strain differentiation or catalytic position in the glycolytic cycle. In the same vein, serum aldolase activity increased in the infected control mice of the susceptible study and this effect was reduced by mefloquine and its supplementation by curcumin. Curcumin supplementation at 25 mg/kg decreased the activity of this enzyme and there was no statistical significance between the effect of mefloquine only and its supplementation with 25 mg/kg curcumin. However, curcumin supplementation at 50 mg/kg was less effective relative to the 25 mg/kg dose (Fig. 3C). Similarly, mefloquine and its supplementation with curcumin decreased serum aldolase although this effect did not vary linearly with curcumin supplementation (Fig. 3D). Infection did not cause significant increase in the activity of glutamate dehydrogenase compared with the normal control especially in the resistant study (Fig. 3F). However, further increase was caused by curcumin (25 mg/kg) supplementation significantly ($P < 0.05$) in the susceptible study (Fig. 3E) but otherwise in the resistant study (Fig. 3F). The effect of curcumin supplementation and treatment was not felt in the resistant study as it was in the susceptible study.

To investigate the modulatory effects of curcumin on immunological balance, we determined the induction of total immunoglobulins G (IgG) and M (IgM) in mouse malaria, in response to blood-stage infection. Here, *Plasmodium* infection decreased the serum concentration of IgG in the infected control and maximum effect of curcumin supplementation on serum increase of IgG level was observed at 25 mg/kg dose ($P < 0.0001$) in both susceptible and resistant studies ($P < 0.0001$ in Fig. 3G and $P < 0.001$ in Fig. 3H). Modulation of serum concentration of IgM by curcumin was presented in Fig. 3I (susceptible) and 3J (resistant studies). It was observed that *Plasmodium* infection caused the serum IgM to decrease, relative to the normal control. Curcumin supplementation of mefloquine treatment increased ($P < 0.01$) IgM level and this increase was found to be linearly correlated with increased ($P < 0.001$) dose of curcumin (Fig. 3J). While similar effect was observed in the susceptible study (Fig. 3I), the extent to which curcumin increased this effect was higher in the susceptible study than the resistant study.

3.4. *Curcumin improves antioxidant status without prejudice to antimalarial properties of mefloquine*

In this study, it was observed that *Plasmodium* infection caused a significant ($P < 0.01$) decrease in catalase activity relative to the normal control. Although, mefloquine increased catalase activity ($P < 0.05$) relative to the infected control, curcumin supplementation at 25 mg/kg ($P < 0.01$) and 50 mg/kg ($P < 0.001$) further increased catalase activity relative to the infected control. In the susceptible study, high dose of curcumin (50 mg/kg) significantly ($P < 0.05$) increased catalase activity

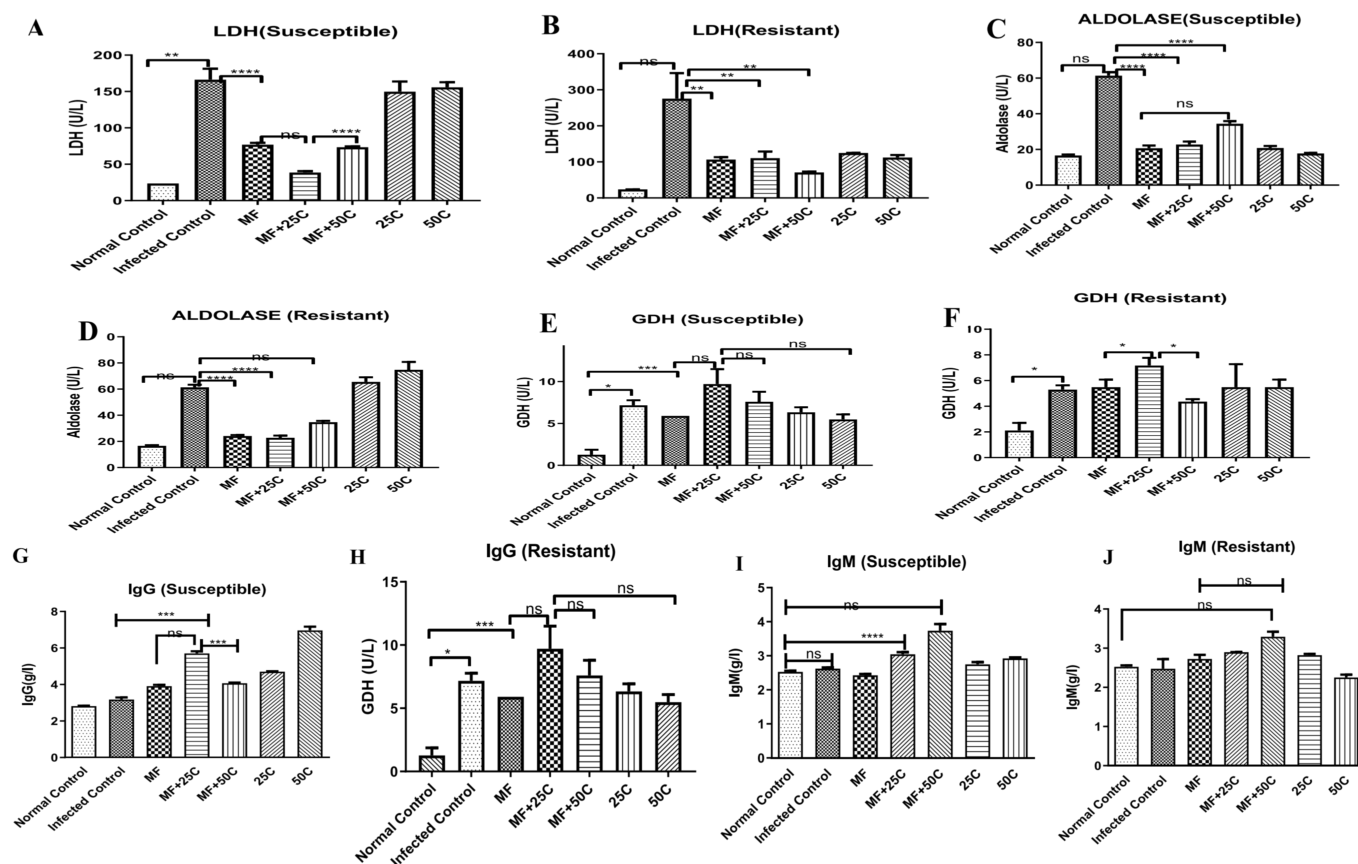

Fig. 3. The effects of mefloquine and curcumin combinations on host glycolytic enzymes (A–F) and immunoglobulins (G–J) in mice infected with susceptible and resistant *P. berghei*-infected. MF = Mefloquine; MF+25C = Mefloquine combined with 25 mg/kg curcumin, MF+50C = Mefloquine combined with 50 mg/kg curcumin; 25C = 25 mg/kg curcumin only; 50C = 50 mg/kg curcumin only. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; **** = $P < 0.0001$.

than the lower dose ($P < 0.01$). Though mefloquine increased catalase activity, curcumin supplementation at both doses increased the activity of this enzyme ($P < 0.05$) than the effect of mefloquine (Fig. 4A). A similar trend of result was obtained in the resistant study; although, curcumin supplementation at 25 mg/kg increased catalase activity albeit insignificantly, 50 mg/kg of this compound supplemented with mefloquine treatment significantly increased catalase activity (Fig. 4B).

The dismutation of superoxides generated by *Plasmodium* infection decreased in the infected control group and curcumin supplementation with mefloquine treatment at 25 mg/kg could not increase the activity of this enzyme in both studies. However, 50 mg/kg curcumin increased superoxide dismutase activity both in the susceptible ($P < 0.001$) and the resistant ($P < 0.01$) studies. Again, curcumin treatment at 50 mg/kg increased superoxide dismutase activity both in the susceptible ($P < 0.01$) and resistant ($P < 0.05$) studies. Although, there was no significant difference between the SOD activity in mice infected with ANKA strain (not treated) and the normal control (see Fig. 4D), a significant difference ($P < 0.01$) was observed when SOD activity in the infected control was compared with the normal control in Fig. 4D.

To further understand the potential benefits of antioxidant to cause resolution of the oxidative stress generated in malaria treatment, the host level of reduced glutathione was estimated. It was observed that *Plasmodium* infection significantly ($P < 0.0001$) decreased the level of reduced glutathione in the host homogenate and there was no significant difference between the levels of GSH in the infected control and mefloquine-treated mice. Supplementation of mefloquine treatment with 25 mg/kg curcumin increased GSH level ($P < 0.05$) and the increase of GSH level by curcumin treatment only was dose-dependent ($P < 0.01$) (Fig. 4E). In the resistant study, mefloquine treatment of mouse malaria decreased GSH level and supplementation of this treatment with 25 mg/kg curcumin increased the level of this tripeptide significantly ($P < 0.0001$) and in the same magnitude, maximally increased the level of GSH than when 50 mg/kg curcumin was used (Fig. 4F).

In order to determine the extent of reduction of lipid peroxides to their corresponding alcohols and oxygen and the reduction of hydrogen peroxide to water and oxygen, the catalytic activity of glutathione peroxidase (GPx) was determined. It was observed that in the susceptible study, *Plasmodium* infection decreased the activity of GPx ($P < 0.001$) compared to the normal control and treatment with mefloquine increased the activity of GPx ($P < 0.01$) relative to the infected control. It was also observed that curcumin supplementation dose-dependently increased the activity of this enzyme ($P < 0.01$). Furthermore, while 25 mg/kg curcumin supplementation increased the activity of GPx ($P < 0.05$), 50 mg/kg further increased ($P < 0.01$) this effect (Fig. 4G). A similar effect was noticed in the resistant study but at different magnitude (Fig. 4H).

To assess the extent of detoxification of the administered xenobiotics, the activity of glutathione S- transferase was determined. It was observed that an increased dose of curcumin (50 mg/kg) supplemented with mefloquine, increased the activity of this enzyme ($P < 0.0001$) in the susceptible study (Fig. 4I). In the chloroquine-resistant study however, there was no significant difference in the activity of this enzyme both in the normal and infected control. The activity of this enzyme in the control groups were higher than mefloquine-treated (normal control vs mefloquine only) and mefloquine-treated, supplemented with 25 mg/kg curcumin (compared with the infected control). Interestingly, supplementation of mefloquine treatment with 50 mg/kg curcumin increased the activity of this enzyme when compared with the infected control, mefloquine treatment and supplementation of mefloquine treatment with 25 mg/kg curcumin ($P < 0.001$, in each case).

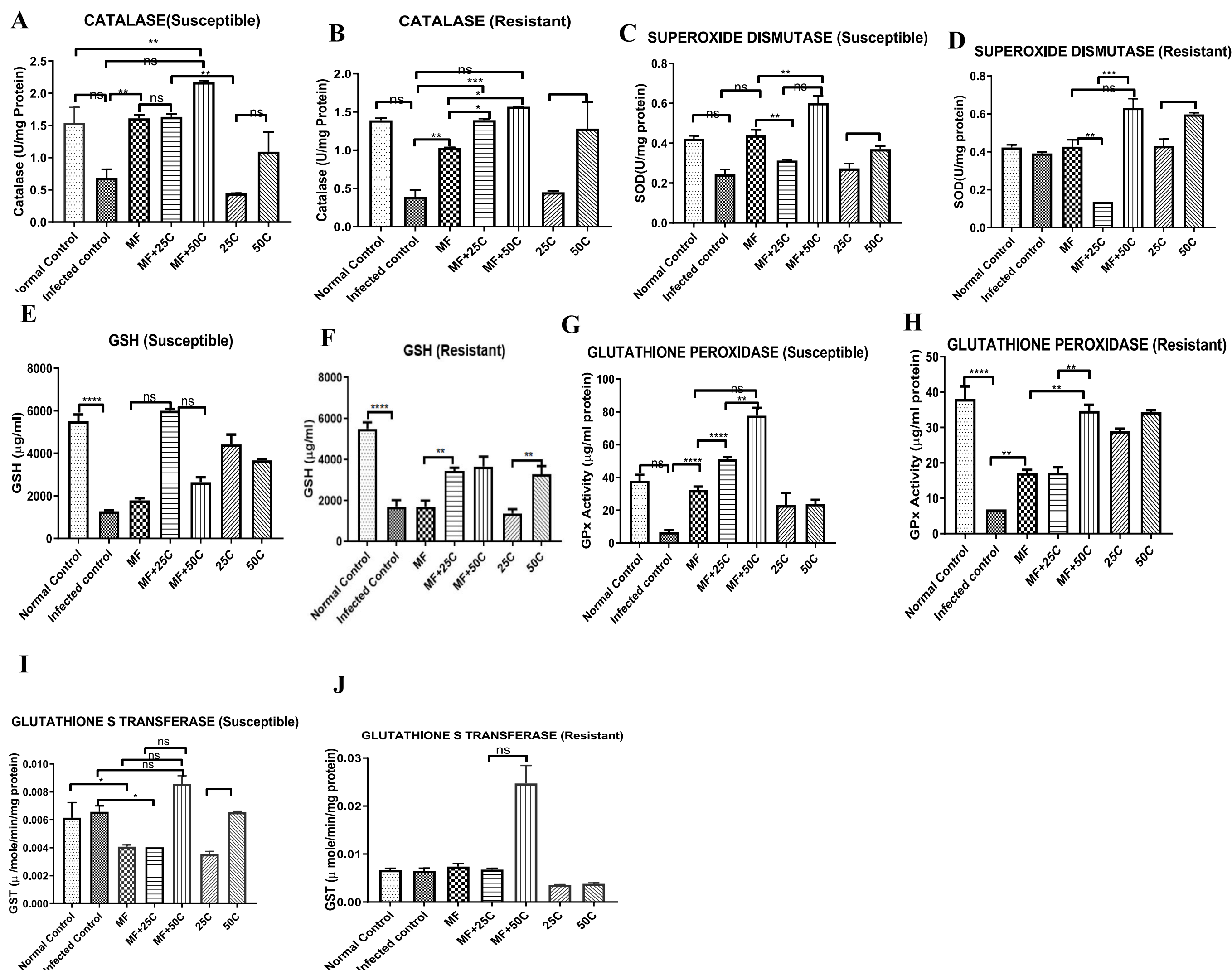

Fig. 4. The effect of mefloquine and curcumin combination on antioxidant systems both in susceptible and resistant studies. MF = Mefloquine; MF+25C = Mefloquine combined with 25 mg/kg curcumin, MF+50C = Mefloquine combined with 50 mg/kg curcumin; 25C = 25 mg/kg curcumin only; 50C = 50 mg/kg curcumin only. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; **** = $P < 0.0001$.

3.5. Curcumin decreases mefloquine toxicity

Toxicity studies of mefloquine was carried out to assess the mitigating effect of curcumin supplementation. It was discovered that using mefloquine to treat mouse malaria significantly ($P < 0.0001$) increased the activity of serum alanine aminotransferase (ALT) both in the susceptible and resistant studies when compared with the infected controls. In the susceptible study, 25 mg/kg curcumin supplementation was found to maximally ($P < 0.001$) decrease the activity of ALT (Fig. 5A). While there was no significant difference in the mitigating effects of the two doses of curcumin supplementation, it was observed that curcumin significantly ($P < 0.0001$) decreased the activity of ALT compared to mefloquine only in the resistant study (Fig. 5B). It was observed also that administration of curcumin only decreased the activity of ALT. Taken together, it was observed that curcumin administration decreased ALT activity. Furthermore, it was discovered that aspartate aminotransferase (AST) activity increased significantly in the infected controls of the two studies. While serum AST in infected mice treated with mefloquine decreased compared with the infected control in the two studies, supplementation with curcumin significantly decreased AST activityt. However, the decreasing effect of curcumin on AST was found not to be dose dependent in the susceptible study where 25 mg/kg curcumin supplementation significantly had the highest ($P < 0.0001$) mitigating effect (Fig. 5C). In the resistant study however, 50 mg/kg curcumin was found to have a higher decrease ($P < 0.001$) in the activity of AST than 25 mg/kg dose of curcumin (Fig. 5D). Mefloquine increased serum gamma glutamyl transferase (GGT) activity than *Plasmodium* infection ($P < 0.01$) in the susceptible study while there was no significant difference in the activity of this enzyme in the resistant study among the two groups considered. Curcumin supplementation (50 mg/kg) decreased GGT ($P < 0.001$) compared with mefloquine in the susceptible study while the 50 mg/kg dose of curcumin decreased the activity of this enzyme than the 25 mg/kg dose albeit, insignificantly (Fig. 5E). In the resistant study, 50 mg/kg curcumin decreased GGT activity ($P < 0.01$) when compared with mefloquine (Fig. 5F).

3.6. Curcumin minimizes necrotic influence of mefloquine on liver and spleen tissues

Liver tissue of the normal mouse appears with no lesion. The representative micrograph showed mild congestion with area of thrombosis occluding the veins, mild disseminated steatosis and infiltration by inflammatory cells (Fig. 6A). In untreated infection by *Plasmodium*, there was marked disseminated bridging periportal infiltration

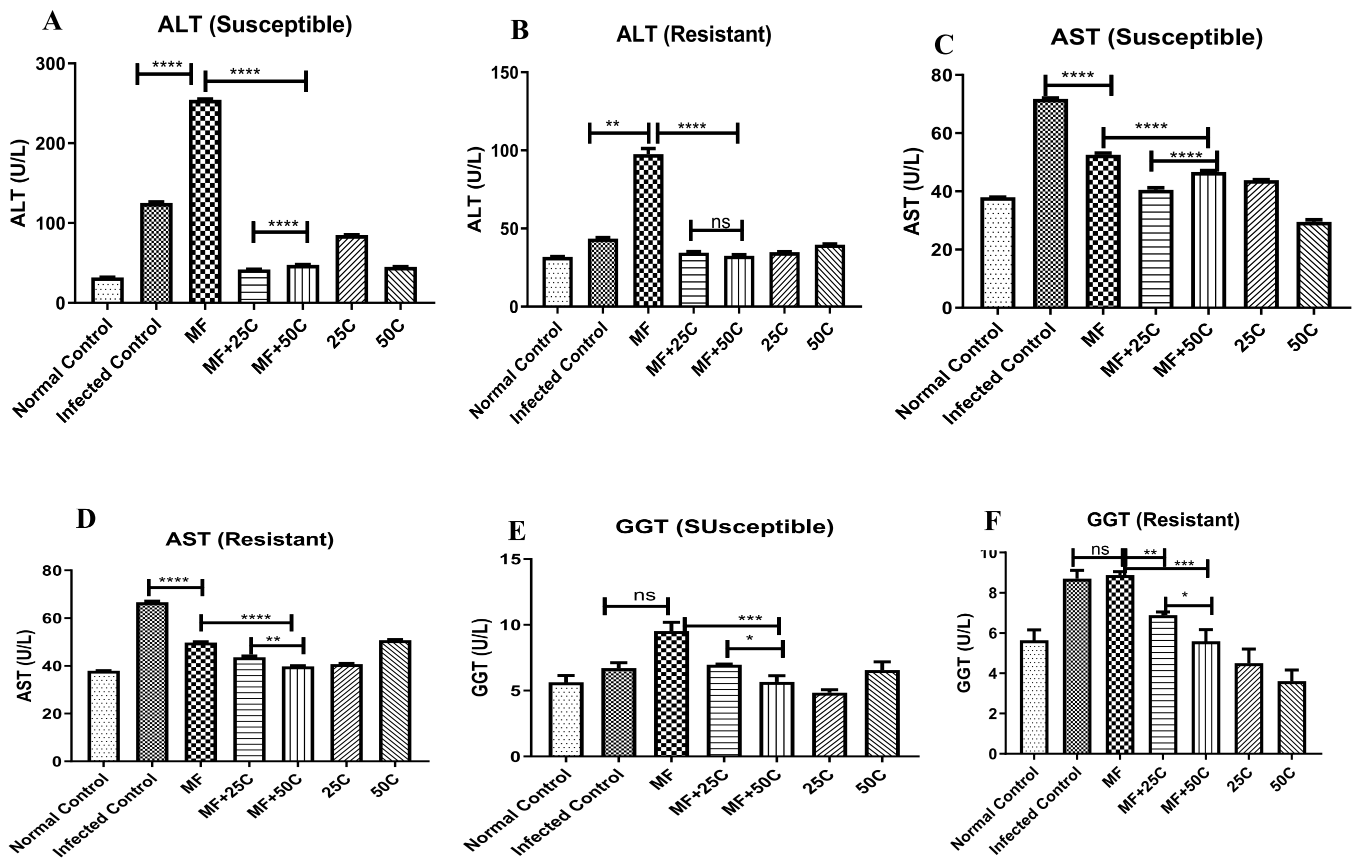

Fig. 5. The effect of mefloquine and curcumin combination on some markers of toxicity. The figures shows alanine aminotraansferase in susceptible (A) and resistant (B); aspartate aminotransferase in susceptible (C) and resistant (D); gamma glutamyl transferase in susceptible (E) and resistant (F) studies. MF = Mefloquine; MF+25C = Mefloquine combined with 25 mg/kg curcumin, MF+50C = Mefloquine combined with 50 mg/kg curcumin; 25C = 25 mg/kg curcumin only; 50C = 50 mg/kg curcumin only. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; **** = $P < 0.0001$.

by inflammatory cells, marked infiltration of zone 2 by inflammatory cells (slender arrows), disseminated steatosis with necrosis (green arrows) (Fig. 6B). When mice infected with *P. berghei* (NK 65 strain) were treated with mefloquine only, there was disseminated congestion and thrombosis, mild disseminated steatosis with necrosis (green arrows) and very mild inflammation of the zone 2 (slender arrows) (Fig. 6C). Supplementation of mefloquine treatment with 25 mg/kg curcumin showed marked disseminated periportal infiltration by inflammatory cells and marked infiltration of zone 2 by inflammatory cells (slender arrows) (Fig. 6D). Supplementation with 50 mg/kg curcumin revealed mild periportal infiltration by inflammatory cells, marked infiltration of zone 2 by inflammatory cells and lymphoid aggregate (slender arrows) and mild disseminated steatosis (green arrows) (Fig. 6E). When mice infected with susceptible *P. berghei* was treated with 25 mg/kg curcumin, there was marked thrombosis and congestion, very mild periportal infiltration by inflammatory cells (black arrows) and marked disseminated steatosis with necrosis (green arrows) (Fig. 6F). Treatment of susceptible mouse malaria with 50 mg/kg curcumin show congestion, marked disseminated bridging periportal infiltration by inflammatory cells, marked infiltration of zone 2 by inflammatory cells and marked to severe disseminated microvesicular steatosis with necrosis (green arrows) (Fig. 6G). In the resistant study, infection by the resistant (ANKA) strain of *P. berghei* caused disseminated microvesicular steatosis (green arrows) and marked infiltration of zone 2 by inflammatory cells (slender arrows) (Fig. 6H).

Mefloquine presented similar toxicity and necrotic cell death (Fig. 6I). Infection by the resistant *Plasmodium* strain presented similar effects on the liver tissue. Liver tissue of mice infected with resistant (ANKA) *P. berghei*, treated with mefloquine supplemented with 25 mg/kg curcumin showed moderate disseminated microvesicular steatosis (green arrows) and focal area of periportal inflammation (black arrows) (Fig. 6J) while supplementation of mefloquine treatment with 50 mg/kg curcumin showed mild periportal infiltration by inflammatory cells (black arrows), moderate disseminated steatosis, mild infiltration by inflammatory cells (black arrows) and mild congestion (blue arrows) (Fig. 6K). The liver tissue of infected mouse treated with 25 mg/kg curcumin show marked disseminated periportal infiltration by inflammatory cells and disseminated microvesicular steatosis (green arrows) (Fig. 6L). Treatment of mouse infection by resistant *P.berghei* with 50 mg/kg curcumin showed mild focal periportal infiltration by inflammatory cells (black arrows), marked infiltration of zone 2 by inflammatory cells (slender arrows) and disseminated steatosis with necrosis (green arrows) of the liver tissue (Fig. 6M). There were no visible lesions in the spleen of mice both in the susceptible and resistant studies. However, well delineated few nodules of white pulp embedded in red pulp, numerous megakaryocytes in the marginal zone of the red pulp and focal area of necrosis (black arrow) were observed in the spleen of infected mice treated with mefloquine only (Fig. 6N).

4. Discussion

The unique serine threonine kinase with a repeating phenylalanine, isoleucine, lysine and lysine (FIKK) in the genome of this phylum of parasite is a family of kinases that modify the host cells and tissues via phosphorylation to suit the survival of the parasites and at the same time causing instability to the host cells (Angel and Linas, 2021). Although, FIKK12 may not mediate virulence as FIKK4.2 does (Kats et al., 2014), both FIKK kinases modify the cytoskeletal; meshwork (proteins) of the

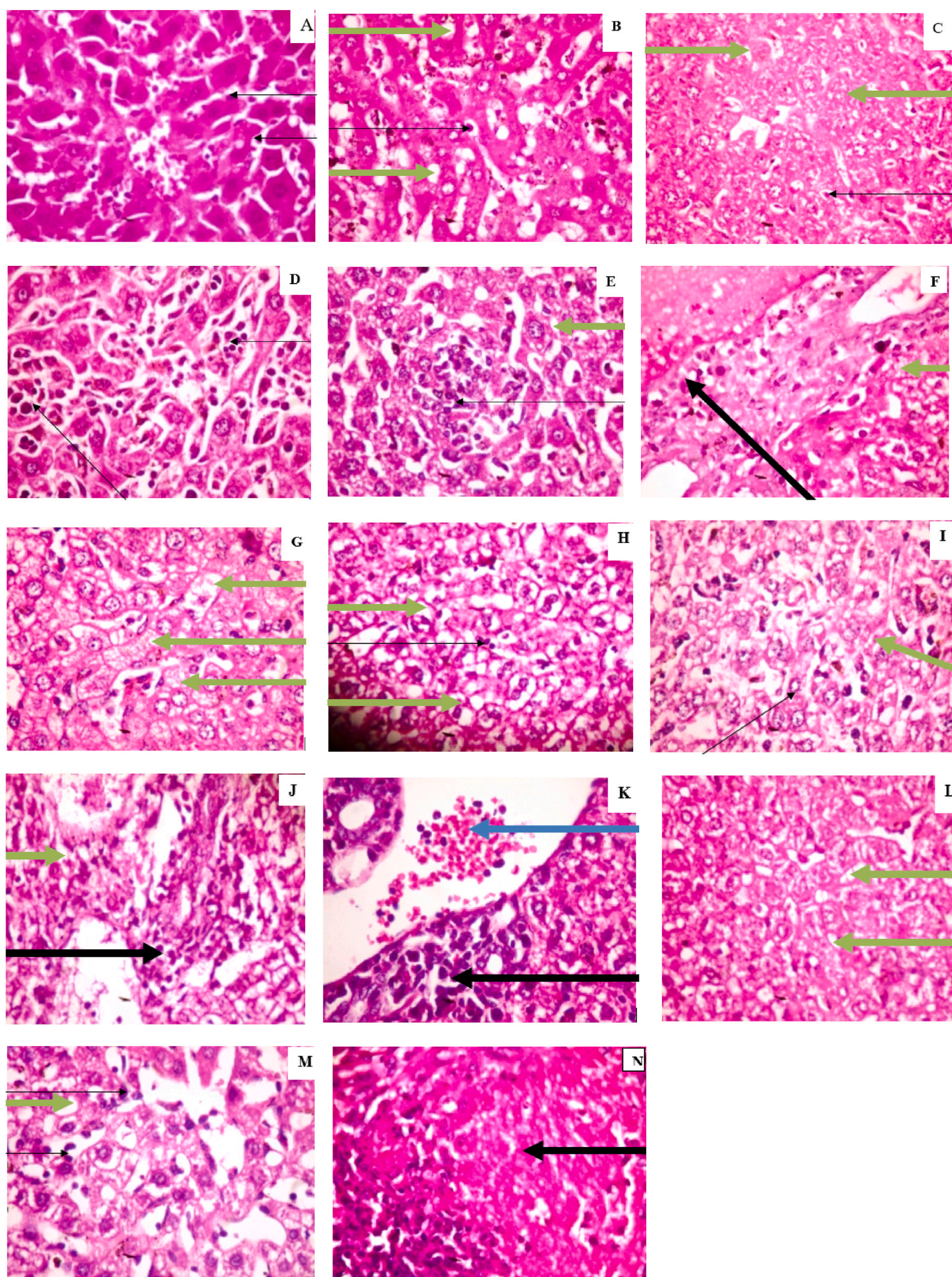

Fig. 6. Histology of liver and spleen of *P. berghei*-infected mice treated with drug combinations of mefloquine and curcumin. A = Normal control; B to G indicates susceptible *P. berghei*-infected mice treated with mefloquine, mefloquine and 25 mg/kg curcumin, mefloquine and 50 mg/kg curcumin, 25 and 50 mg/kg of curcumin only. H to M indicates resistant *P. berghei*-infected mice treated with vehicle, mefloquine, mefloquine combined with 25 mg/kg curcumin, mefloquine combined with 50 mg/kg curcumin, 25 and 50 mg/kg of curcumin only. The plate labeled N is the spleen from infected mice treated with mefloquine only.

infected erythrocytes thus rendering them to be unstable via their phosphorylation. Specifically, the phosphorylation of band 4.1 reduces its affinity for spectrin thus causing instability of the cytoskeletal meshwork of the erythrocyte membrane making it susceptible to lysis (Nunes et al., 2010). It is interesting to note therefore, that as curcumin supplementation decreases the phosphorylation of erythrocyte membrane proteins, it enhances their stability, alters their remodeling and significantly decrease cyto-adhesion. A previous study has found that

curcumin interacts with erythrocyte ghosts perhaps as a protective shield against phosphorylation or peroxidation of its membrane lipids (Sneharani, 2020). The biphasic response observed in this study could be that the supplementary and sole effects of curcumin is dose dependent as observed in a similar previous study (Singh and Rizvi, 2015).

In the cause of parasite invasion, host machinery are used to favour parasite survival. In addition, some host genes are up-regulated in response to parasite load and are down-regulated as a symbol of clearance. A previous study has reported the up-regulation of aquaporins 3 and 9 genes up-regulation during *Plasmodium* infection (Posfai et al., 2018). It was observed in this study that mefloquine as AQP3 inhibitor and by extension, supplementation with curcumin further enhanced the inhibition of this gene. It shows that mefloquine decreases the expression of this gene in the liver of the host thus decreasing host-parasite interactions in both dormant and replicating liver stage of *Plasmodium berghei.* Basically, some of these aquaporin transmembrane proteins are required for entry of sporozoites into the hepatocytes (Amanzougaghene et al., 2021). However, it must be stressed that the AQP3 gene was silenced by mefloquine and a more significant episode was observed in the presence of curcumin because AQP3 knockout in mice have been found not to protect against malaria and may cause renal duct abnormalities and worsens ischemia reperfusion injury (Lei et al., 2017).

Malarial parasite uses various host signaling pathways to favour its survival and one such pathway is P38 mitogen activated protein kinase (MAPK) signaling pathway. In this study, the effect of curcumin supplementation on mefloquine treatment in aquaporin-3 and MAPK in *P. berghei*-infected malaria are related as with other study (Sicard et al., 2011). The silencing of AQP3 affects MAPK signaling as there was a corresponding decrease in the expression of the gene for this signaling molecule. Previously, host erythrocyte signaling molecules have been implicated in parasite survival (Brumlik et al., 2011). This further shows that as the mefloquine-curcumin combination inhibits MAPK and effectively controls the proliferation of infected erythrocytes and the invading parasite, they further commute the infected cells to death via apoptosis. Our identification of mefloquine as a MAPK inhibitor further corroborate a previous study where this drug was earlier identified as a MAPK inhibitor (Erdman et al., 2009). It has been shown previously that CD36 can induce MAPK leading to an increase in the production of TNF-α and induction of cytokine responses in mouse cells (Patel et al., 2007; Erdman et al., 2009; Gowda et al., 2013; Olanlokun et al., 2022). Our observation in this study, that mefloquine and curcumin supplementation abrogates MAPK signaling further showed that this drug combination will inhibit inflammatory cytokine production which is a pathological event in *Plasmodium* infection (Cumnock et al., 2018).

Energy generation in *Plasmodium* infected host is an onerous task as a result of competition between the parasite and the host. For quick recovery from malaria, associated pathological effects, disease tolerance and host energy source are very important (Schimo et al., 2017). Results from this study showed that *Plasmodium* infection in both studies decreased NADH oxidoreductase and cytochrome oxidase, suggesting that mouse malaria leads to mitochondrial damage and decrease in oxidative phosphorylation in the host cell. Furthermore, recovery was found to be significant with mefloquine treatment and dependent on combinative treatment and doses of curcumin. The relatively low or null expression of the *NADH* oxidoreductase gene in *Plasmodium* infection in the resistant study further confirmed that indeed, *Plasmodium* infection affects the expression of this gene and that such effect is not strain dependent.

Full recovery for bioenergetics function via the respiratory activity of NADH oxidoreductase by the administration of curcumin showed that curcumin possesses a reversal effect against *Plasmodium* damage of mitochondria. Cytochrome oxidase is the terminal acceptor of electrons in the mitochondrial respiratory chain. The efficiency of this protein enhances bioenergetics functions of the organelle and efficient transfer of electron to oxygen to prevent leakage and resultant oxidative stress (Boutlis et al., 2017). It is possible that nitric oxide synthesis, stimulated by *Plasmodium* infection (Cooper and Brown, 2008) inhibits the expression of cytochrome oxidase gene (Chaurasiya et al., 2021) and may be the reason for the silencing of the cytochrome oxidase gene caused by *Plasmodium* infection. A recent finding showed that inhibition of glycolytic enzymes is a key feature of new antimalarial drugs (Jezewski et al., 2021). A previous study has identified the targeting of protein synthesis in *P. falciparum* as a major mechanism of action of mefloquine (Wong et al., 2017). However, in addition to this, we identified the inhibition of host lactate dehydrogenase by mefloquine as one of its mechanistic roles in malaria chemotherapy both in the parasite and in the host. The understanding that this enzyme is favourably inhibited in the parasite by decreasing the amount of energy generated via glycolysis further shows that ATP generation in the host via mitochondrial respiration is impeded because of the decrease in the amount of NADH, an important substrate in oxidative phosphorylation (Lemire et al., 2008). Further to this, a further decrease in the expression of *NADH* oxidoreductase in the infected control coupled with decreased NADH/NAD + ration in favour of NADH typifies bio-energetic crisis expected in untreated malaria. Therefore, the inhibition of pathogen-mediated host NAD^+ modulation is critical for the regulation of infection (Starnes et al., 2009). Aldolase is a critical enzyme that converts a ketose sugar to aldose sugar in the glycolytic pathway. It bridges the parasite adhesion and actin of the host cell component for successful invasion apart from the energy functions (Romaea et al., 2022). The inhibition of host aldolase activity by mefloquine and further by curcumin supplementation as observed in this study showed that this event will possibly lead to the detachment of parasite adhesion from the cytoskeletal actin in order to ensure a successful invasion of the host cell by the parasite. A previous study has shown that curcumin is an active inhibitor of aldolase and other glycolytic enzymes in the host cell (MacRae et al., 2013). Therefore, supplementation of curcumin with mefloquine treatment of mouse malaria may be suggested as a good combination for the successful treatment of malaria.

Glutamate dehydrogenase (GDH) is a mitochondrial enzyme and it is currently used as a marker for mitotoxicity. We have previously shown that treatment of malaria using mefloquine only has mitotoxic effect through the opening of the mitochondrial permeability transition pore mefloquine (Church et al., 2020; Olanlokun et al., 2022). Previous observation has shown that damaged mitochondria release their contents into the cytosol (McGill and Jaesche, 2021). Therefore, dysfunction of mitochondria will increase the serum level of glutamate dehydrogenase and perturbs the glutamate/oxaloacetate conversion in the cells. Decrease in the host and pathogens GDH depend on glucose and glutamine for energy production. *Plasmodium*-infected red blood cells and immune cells of the host depends on glutamine, among others, which is channeled to the tricarboxylic acid cycle for ATP production (O'Neill et al., 2016; Vijay et al., 2020; Wanjala et al., 2020). However, the inhibition of glutamine metabolism is associated with increased survival in a murine model of late stage cerebra-malaria by reducing immune-mediated pathology in the brain (Bruguera and Herrera, 2007). The relationship between pre-existing immunity and drug treatment in symptomatic malaria has been established. (Bruguera and Herrera, 2007). Most research on immunity against malaria have targeted immunoglobulin G (IgG), the human antibody that targets *Plasmodium* merozoites. However, the commendable roles of immunoglobulin M (IgM) in protective immunity has received limited attention. The mechanism by which mefloquine treatment supplemented with curcumin significantly increased IgG and IgM titres as observed in this study is not known. Interestingly, a previous study has shown that curcumin stimulates primary and secondary humoral immune antibody titres (Afolayan et al., 2018). Curcumin has previously been combined with some antimalarial drugs (Curcumin-Artesunate and Curcumin-Piperine) and has also been documented as an immunomodulatory agent (Oyeyemi et al., 2018; Khairani et al., 2021). Although, the anti-plasmodial mechanism of action of curcumin could not be categorically esttablished; studies have shown that high dose of curcumin inhibited

iron absorption thus decreasing the survival of malaria parasite (Chin et al., 2014; Cory et al., 2018; Samba-Mondonga et al., 2019).

The observation in this study that the antioxidant system of the treated mice improved show that antioxidants may be needed in malaria chemotherapy especially to prevent complications and prevalence (Zeba et al., 2008).

Drug toxicity is a major factor for their withdrawal. In this study, a therapeutic dose of mefloquine increased the serum activities of AST, ALT and GGT which may lead to acute hepatitis as previously observed (Croft and Herxheimer, 2002). Although, the mechanism of this adverse effect of mefloquine is currently unknown, it could be linked to free radical-mediated toxicity. In addition, it could be that in the cause of its extensive metabolism in the liver, mefloquine metabolites may be toxic or induce immune-mediated hepatotoxicity. The absence of this toxicity when curcumin was administered indicates the plausibility of the free radial-mediated toxicity theory.

The combination of the splenic macrophage phagocytic system, chemotherapy and the protective roles of antibodies show that multiple balances determine the outcome of malaria infections. It was observed that co-administration of curcumin with mefloquine prevented organ damage. The dose-dependent response in relation to the effect of curcumin supplementation was also observed. Adverse effects for the administration of curcumin at the highest dose either singly or in combination with mefloquine was not observed. The protective effect of curcumin could be as a result of its antioxidant property and usage at the tolerated doses.

5. Conclusion

In conclusion, this study showed that treatment of resistant mouse malaria using mefloquine and curcumin provides a better effect on the molecular mechanism of action and improves mitochondrial respiration in the host. Assay of FIKK 12 and Aquaporin-3 have shown that mefloquine can be supplemented with curcumin to enhance mitochondrial respiration without prejudice to its antiplasmodial effects. Therefore, curcumin supplementation with mefloquine can reversibly silence the expression of these genes to regulate infection. It is therefore, pertinent to say that curcumin supplementation in malaria chemotherapy is essential for a better outcome of treatment.

CRediT AUTHOR STATEMENT

We authors hereby state that: JO conceived this research idea, did the lab work, perform assays and wrote the draft manuscript; WO treated the experimental animals, perform assays and did the statistical analysis; NA provided equipment, read the draft manuscript; OO read the draft manuscript. We authorise the publisher to share an accurate and detailed ***description of our research if eventually accepted for publication.***

Ethical approval

This study was carried out in accordance with the ARRIVE guidelines. This study was approved by University of Ibadan Animal Care and Use for Research Ethics Committee (ACUREC) and an approval number UI-ACUREC/05/12/2022A was assigned to the study.

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Availability of data and materials

This declaration is not applicable.

Declaration of competing interest

Authors declare that no competing interest exists.

Data availability

The data that has been used is confidential.

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