

Original article:

**IN VITRO STUDY OF PARASITE ELIMINATION AND
ENDOTHELIAL PROTECTION BY CURCUMIN:
ADJUNCTIVE THERAPY FOR CEREBRAL MALARIA**

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ABSTRACT

Plasmodium falciparum infection can abruptly progress to severe malaria and cerebral malaria. Despite the current efficiency of antimalarial drugs in killing parasites, no specific effective treatment has been found for cerebral malaria. Thus, a new strategy targeting both parasite elimination and endothelial cell protection is urgently needed in this field. In this study, we determined whether curcumin, which has blood-brain permeability, antioxidative activity and/or immunomodulation property, provided a potential effect on both parasite elimination and endothelial protection. Murine brain microvascular endothelial cells (bEnd.3; ATCC) were cocultured with *Plasmodium falciparum*-infected red blood cells (Pf-IRBC), peripheral blood mononuclear cell (PBMC) and platelets. Apoptosis of endothelial cells was demonstrated by annexin V staining. Interestingly, curcumin exhibited high efficiency of antimalarial activity (IC₅₀ ~10 μM) and decreased bEnd.3 apoptosis down to 60.0 % and 79.6 % upon pre-treatment and co-treatment, respectively, with Pf-IRBC, platelets and PBMC. Our findings open up a high feasibility of applying curcumin as a potential adjunctive compound for cerebral malaria treatment in the future.

Keywords: apoptosis, brain endothelial cells, curcumin, malaria, *Plasmodium falciparum*

INTRODUCTION

Cerebral malaria (CM) accounts for a significant proportion of the morbidity and mortality associated with malaria in children with the age of less than five years. Despite a massive amount of experimental

and clinical works, the pathophysiologic mechanisms of such complication are poorly understood. Pathological studies in Southeast Asian adults dying from CM had shown a definitive association between sequestration of *Plasmodium falciparum*-infected red blood cell (Pf-IRBC) in the

brain and the coma resulted from CM sequestration (Silamut et al., 1999).

The symptoms of CM range from confusion or stupor to obtundation, convulsions and deep coma with long-term neurological deficits such as cortical blindness. CM is associated with activation of immune cells and anticoagulant platelets, elevated pro-inflammatory cytokine levels, and endothelial damage (Miller et al., 1994).

Undoubtedly, interaction between *P. falciparum* and the human host cells during malarial infection leads to pathogenesis of the disease. Then, parasite elimination is more beneficial in controlling the pathological condition. Conventional emergency treatment is based on intravenous administration of antimalarial drugs such as quinine and artemisinin derivatives (van Hensbroek et al., 1996). Artemisinin derivatives have many advantages over quinine, most notably a far better safety profile, with fewer serious side effects. A large multicenter, multi-country, open-label randomized clinical trial on the treatment of malaria in Southeast Asia definitively showed decreased mortality with artesunate (15 %) as compared with quinine (22 %) treatment (Dondorp et al., 2005). However, the use of antimalarial drugs such as artesunate, although effective in global malaria control programs, is interrupted by high cost and limited supply (Butler, 2004). Therefore, identification of an antimalarial drug that is easy to produce, inexpensive, and low toxicity across a diverse population represents the ideal agent needed for global malaria control programs.

Curcumin (diferuloylmethane) is a natural polyphenolic compound in the rhizome of the perennial herb turmeric, *Curcuma longa* Linnaeus. It is widely used as a dietary spice and coloring in cooking and as a herb in traditional Indian medicine (Sharma et al., 2005). Curcumin exhibits a wide range of pharmacological activities, including anti-inflammatory, anti-carcinogenic, and anti-infectious activities. The anti-inflammatory and anti-carcinogenic effects

of curcumin largely depend on its antioxidant activity. In addition, curcumin possesses potential activities against bacteria, fungi, and protozoa. Cytotoxic and parasitocidal effects of curcumin on protozoan parasites have been demonstrated in cultures against *Leishmania*, *Trypanosoma*, *Giardia*, and *Plasmodium falciparum* (Koide et al., 2002; Nose et al., 1998; Perez-Arriaga et al., 2006; Rasmussen et al., 2000; Saleheen et al., 2002). In vivo, curcumin also displayed potent activity against *Plasmodium berghei*, and it was synergistic with artemisinin (Reddy et al., 2005). However, study of curcumin on the combining parasite killing and endothelial protection has not been elucidated yet.

Herein, we investigated the effect of curcumin on parasite elimination and endothelial protection using in vitro systems. Two approaches were used:

- 1) antimalarial activity; and
- 2) anti-apoptosis of curcumin upon brain endothelial cells exposure to *Plasmodium falciparum*-infected red blood cell (Pf-IRBC) and influential factors (immune cells and platelets).

MATERIALS AND METHODS

Reagent

Curcumin (Sigma Chemical, St. Louis, MO) was dissolved in 100 % dimethyl sulfoxide (DMSO; Sigma Chemical) and stored in aliquots at -20 °C. Tocopherol was a generous gift from Prof. Supaluk Prachayasittikul (Centre of Data Mining and Biomedical Informatics, Faculty of Medical Technology, Mahidol University).

Plasmodium falciparum culture

The *Plasmodium falciparum* strain TM-267 was a generous gift from Prof. Rachanee Udomsangpetch (Department of Pathobiology, Faculty of Science, Mahidol University). *Plasmodium falciparum*-infected red blood cells (Pf-IRBC) were cultured in RPMI-1640 medium containing 10 % human serum (HS), as described previously (Trager and Jensen, 1976). The par-

asite culture was maintained at 37 °C with 5 % CO₂. Briefly, sorbitol-synchronized trophozoite-stage Pf-IRBC was purified using Percoll density-gradient centrifugation (Lambros and Vanderberg, 1979). Parasitemia was then adjusted to 50 % Pf-IRBC and hema-tocrit to 1, 3 and 5 % Hematocrit (Hct). The term of 50 % Pf-IRBC (or 50 % parasitemia) corresponds to approximately 5-50 parasitized erythrocytes/HBMEC sequestered in the microvasculature during human infections (MacPherson et al., 1985; Pongponratn et al., 1985; Silamut et al., 1999).

Brain endothelial cell culture

Murine brain microvascular endothelial cells (bEnd.3; ATCC) were cultured in DMEM medium supplemented with 10 % fetal bovine serum, 0.7 mM L-glutamine, 100 µg/ml penicillin, 100 U/ml streptomycin, and 100 µg/ml gentamicin. The bEnd.3 cells were maintained at 37 °C with 5 % CO₂ before adjustment to 7.0×10^5 cells/cm² in 25 cm² culture flasks (Costar) for further experiment.

In vitro antimalarial assay on blood stage of *P. falciparum*

Antimalarial activity of curcumin was evaluated against *P. falciparum* chloroquine resistant strain (TM 267) using the well-accepted method (Satayavivad et al., 2004). *P. falciparum* culture was synchronized by using sorbitol-induced hemolysis according to the method of Lambros and Vanderberg (1979) to obtain ring stage-infected red blood cells and then incubated for 48 hours prior to the drug testing to avoid the effect of sorbitol. The experiments were started with synchronized suspension of 2 % Pf-IRBC during ring stage. Parasites were suspended with culture medium supplemented with 15 % human serum to obtain 10 % cell suspension. The parasite suspension was put onto a 96-well microculture plate; 50 µL in each well and then added 50 µL of various concentrations of curcumin as follows: 0.01, 0.1, 1, 10 and 100 µM. The rate

of parasite growth was observed for 4 days and each concentration was performed in triplicate. The percentage of parasitemia of control and curcumin-treated groups were examined by microscopic technique using methanol-fixed Giemsa stained thin smear blood preparation. The efficacy of curcumin was evaluated by determining the concentration that reduced the growth of parasite by 50 % (IC₅₀). Antimalarial drug; artesunate was used as positive control (Wongsrichanalai et al., 1999).

Cytotoxic assay of curcumin

Cytotoxic assay of curcumin was performed using the modified method as previously described (Tengchaisri et al., 1998). The bEnd.3 cells were cultured in DMEM medium supplemented with 10 % fetal bovine serum, 0.7 mM L-glutamine, 100 µg/ml penicillin, 100 U/ml streptomycin, and 100 µg/ml gentamicin. Briefly, bEnd.3 cells were seeded at 1×10^4 cells (100 µL) per well in a 96-well plate, and incubated in humidified atmosphere, 95 % air, 37 °C, 5 % CO₂ until they reached 100 % confluence. The bEnd.3 cells were incubated for 72 hours with various concentrations of curcumin. Then, cells were incubated with MTT solution (1 mg/ml) for 4 hours at 37 °C, 5 % CO₂. After that, formazan-salt generated by mitochondria of viable cells as results of conversion of MTT was dissolved in 100 % DMSO and the absorbance was measured at 540 nm. IC₅₀ values were determined as the bioactive compounds concentrations at 50 % inhibition of the cell growth.

Preparation of peripheral blood mononuclear cells

This study was obtained IRB approval (MU-IRB 2012/058.2703) from the Committee on Human Rights Related to Human Experimentation, Mahidol University, Bangkok, Thailand. Informed consent was obtained from each individual before samples were taken. Peripheral blood from a healthy volunteer was collected in a sterile

tube containing heparin solution. The blood was mixed with phosphate-buffered saline (PBS) at a ratio of 1:1, followed by over layering on Histopaque 1077° (Sigma-Aldrich, USA) and centrifugation at 400 g for 30 minutes. Peripheral blood mononuclear cells (PBMC) were collected from the interface, washed twice with PBS at 250 g for 10 minutes, and resuspended in DMEM medium supplemented with 10 % fetal bovine serum, 0.7 mM L-glutamine, 100 µg/ml penicillin, 100 U/ml streptomycin, and 100 µg/ml gentamicin. The viability of PBMC was determined using a 0.2 % (w/v) trypan blue dye exclusion assay. All experiments were performed at a PBMC viability of over 90 %. The PBMC were maintained in RPMI-1640 medium before adjustment to an optimal ratio of PBMC to bEnd.3 cells at 7.0×10^5 cells/cm² to 7.0×10^5 cells/cm². This ratio did not show any affect to bEnd.3 cells for co-culturing, observed by AnnexinV assay (flow cytometry).

Preparation of platelets

Peripheral blood from a healthy volunteer was collected in sterile BD Vacutainer tubes (BD Biosciences, San Jose, CA, USA) containing 0.32 % sodium citrate. Platelet-rich plasma (PRP) was prepared by centrifugation at 200 g for 15 minutes at room temperature, and platelets were then pelleted by centrifugation of the PRP at 2,000 g for 6 minutes. Following washing in prewarmed Tyrode buffer salt solution (TBSS), the platelets were maintained in the same buffer before adjustment to an optimal ratio of platelets to bEnd.3 cells at 1.0×10^9 cells/cm² to 7.0×10^5 cells/cm². This ratio did not show any effect to bEnd.3 cells for co-culturing, observed by AnnexinV assay (flow cytometry).

Apoptosis assay

Parasitemia level and incubation period

To assess the effects of Pf-IRBC on bEnd.3 cells apoptosis, cells were cultured (7.0×10^5 cells/cm²) in 25 cm² culture flasks (Costar). The Pf-IRBC (trophozoite-stage)

at 1, 3 and 5 % Hct, 50 % Pf-IRBC were added and incubated with the cells for 4 and 20 hours in parasite culture medium. In control, the bEnd.3 cells were incubated with uninfected RBC (5 % Hct, uninfected RBC). After incubation, all factors were removed and the cells were washed twice with cold PBS and then resuspended in 1X binding buffer at a concentration of 1×10^6 cells/ml. Then, 100 µl of the solution (1×10^5 cells) were transferred to a 5 ml culture tube. Annexin V-Alexa488/Propidium iodide (PI) was added to the solution. The solution was gently mixed with the cells and further incubated for 15 minutes at room temperature in the dark. The binding buffer was added to each tube at a volume of 400 µl. The apoptotic cells were analyzed by flow cytometry within 1 hour.

Parasitemia and influential factors (immune cell and platelets)

Since, CM involved with host responses such as activation of immune cells, inflammation and interrupting blood coagulation/thrombosis (Hunt et al., 2006; Miller et al., 2013; Miller et al., 1994; Turner 1997), we tested whether supplementation with immune cells (PBMC) and platelets would affect the viability of brain endothelial cells.

The lowest-observed-adverse-effect level (LOAEL) of Pf-IRBC that causes an adverse alteration of cell viability was chosen for this experiment. All factors: Pf-IRBC, PBMC and platelets at: LOAEL of Pf-IRBC (% Hct, 50 % Pf-IRBC), 7.0×10^5 cells/cm² and 1×10^9 cells/cm², respectively were added and incubated with the bEnd.3 cells for 4 and 20 hours in parasite culture medium. In control, the bEnd.3 cells were incubated with uninfected RBC, PBMC and platelets at the same dose for 4 and 20 hours. After incubation, all factors were removed and cells apoptosis was analyzed as abovementioned.

Effect of curcumin on endothelial protection

To verify the capacity of curcumin on endothelial protection upon exposure to Pf-IRBC and influential factors (immune cells and platelets), curcumin at IC_{50} (μM) was added to bEnd.3 cells cultivation in the pre-treatment and co-treatment experiments.

The efficiency of curcumin was compared with well-known antioxidant compound. In this case, tocopherol was chosen due to its capacity in modulating the effect of malarial infection (Adelekan et al., 1997; Das et al., 1996). The concentration of tocopherol ($\sim 10 \mu M$) was used to reflect level in serum that can be analyzed under clinical condition of severe malaria (Das et al., 1996). Antimalarial drug; artesunate at IC_{50} (nM) was used as positive control.

Statistical analysis

For quantitative analysis, all data were expressed as means \pm SD. The significance of differences in the data was evaluated by the Mann-Whitney U tests. Correlation was calculated as Spearman rank correlations. Statistical significance was evidenced at $p < 0.05$.

RESULTS

Antimalarial activity of curcumin

Antimalarial activity of curcumin was evaluated against *P. falciparum* chloroquine resistant strain (TM 267) compared with artesunate (served as a positive control). Our study showed that the artesunate inhibited parasite growth at ~ 10 nM, which is in good agreement with the previous study (Wongsrichanalai et al., 1999). Interestingly, curcumin inhibited the growth of *P. falciparum* in a dose-dependent fashion; with an IC_{50} of $\sim 10 \mu M$ (Figure 1).

Cytotoxicity testing of curcumin

The effects of curcumin on bEnd.3 cells were assessed to more than 95 % viability as confirmed by trypan blue exclusion assay. To identify the optimal concentration of curcumin, MTT assay was performed. In

this study, DMSO was used as a solvent for curcumin, which did not show any cytotoxic effects. When curcumin was applied to cultured bEnd.3 cells for 72 hours, cells were almost 100 % viable in the presence of curcumin up to a concentration of 100 μM . However, above this concentration, the cell viability showed less than 95 % (Figure 2).

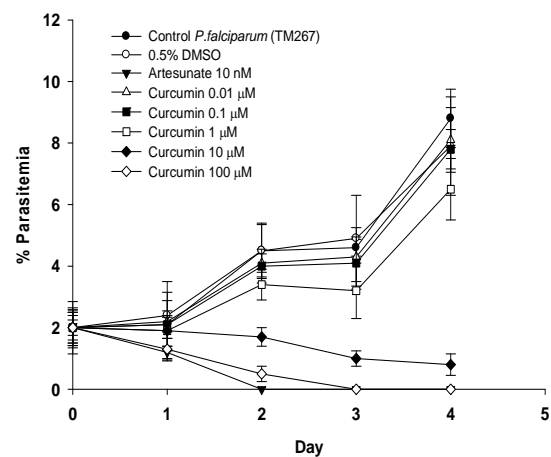


Figure 1: In vitro antimalarial activity of curcumin. Assays were done in triplicate and averages from experiments are shown. Error bars indicate standard deviation.

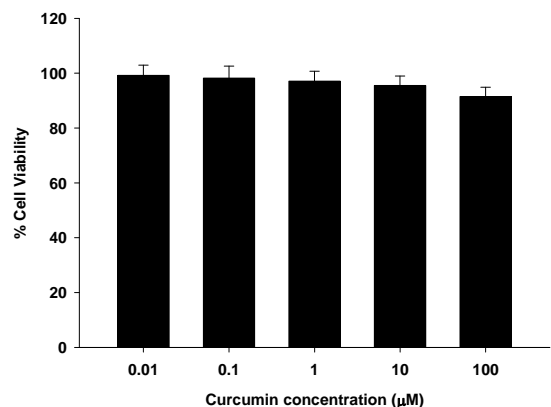


Figure 2: Cytotoxicity testing of curcumin. These data were representative of three independent experiments that yielded similar results.

Role of parasitemia and incubation period on brain endothelial cell apoptosis

To assess the effect of parasitemia and incubation period on brain endothelial apoptosis, Pf-IRBC (1, 3 and 5 % Hct, 50 % Pf-IRBC) at trophozoite-stage, were added to bEnd.3 cells grown in 25 cm² culture flasks for 4 and 20 hours. Annexin V staining revealed that apoptosis of bEnd.3 cells was remarkably observed after 20 hours incubation with Pf-IRBC compared with 4 hours (Figure 3). Such evidences supported that the intensity of bEnd.3 cells apoptosis increased with time. Additionally, parasitemia levels induced a significant correlation with the percentage of bEnd.3 cells apoptosis ($r = 0.97$, $p < 0.001$). Our data indicated that Pf-IRBC induced bEnd.3 cells apoptosis depended on parasitemia level and incubation period (Table 1).

Role of parasitemia and influential factors (immune cell and platelets) on brain endothelial cell apoptosis

The lowest-observed-adverse-effect level (LOAEL) of Pf-IRBC that caused an adverse alteration of cell viability to apoptosis at 1 % Hct, 50 % Pf-IRBC was chosen for this experiment. Influential factors such as immune cell (PBMC) and platelets that involved with brain endothelial alteration in CM were chosen for in vitro study.

Double-labeling Annexin V and Propidium iodide showed that 0.95 % of annexin V–Alexa 488–positive cells and 7.90 % of double Annexin V and Propidium iodide–positive cells were found in bEnd.3 cells cocultivated with Pf-IRBC, platelets and PBMC, whereas only 0.16 % and 0.07 % was detected in control bEnd.3 cells cocultivated with uninfected RBC, platelets and PBMC (Figure 4). Our results demonstrated that increasing of bEnd.3 cells apoptosis depended on a synergistic effect between parasitemia and host cells; platelets and PBMC (Table 2).

Effect of curcumin on endothelial protection

Capacity of curcumin on endothelial protection against the consequence effect of cell apoptosis in the presence of Pf-IRBC, platelets and PBMC (control experiment) was evaluated by pre-treatment and co-treatment with curcumin (10 μM) compared with tocopherol (10 μM), while artesunate (10 nM) was used as positive control. After 20 hours incubation, it was found that artesunate inhibited Pf-IRBC, platelets and PBMC induced bEnd.3 cell apoptosis from 10.4 % to 1.93 % and 0.41 % of pre-treatment and co-treatment, respectively. Interestingly, curcumin exhibited great efficiency of endothelial protection in which reduction of endothelial cells apoptosis was detected from 10.4 % to 4.16 % and 2.12 % on pre-treatment and co-treatment, respectively, while tocopherol had lower protective effect (6.32 % and 7.68 % on pre-treatment and co-treatment, respectively) as compared to the control experiment (Table 3). Our data showed great efficiency of curcumin on endothelial protection upon cultivation with Pf-IRBC, platelets and PBMC, which reduced bEnd.3 cells apoptosis at 60.0 and 79.6 % of pre-treatment and co-treatment, respectively, compared with the artesunate (81.4 and 96.1 %) (Table 3).

DISCUSSION

Antimalarial drugs are the cornerstone of malaria treatment; however, even the most used parasite-killing drug such as artesunate, could not prevent deaths among children with severe complications of malaria, such as severe anemia (1 % mortality) (Marsh et al., 1995), acidosis (15 % mortality) or coma (18 % mortality) (Dondorp et al., 2010). Thus, rapid and effective parasite killing is insufficient to prevent death in severe malaria.

Beyond mechanically obstructing small vessels, adherent infected erythrocytes transduce pathologic endothelial activation signals that promote adhesion, coagulation and inflammation, and disrupt endothelial

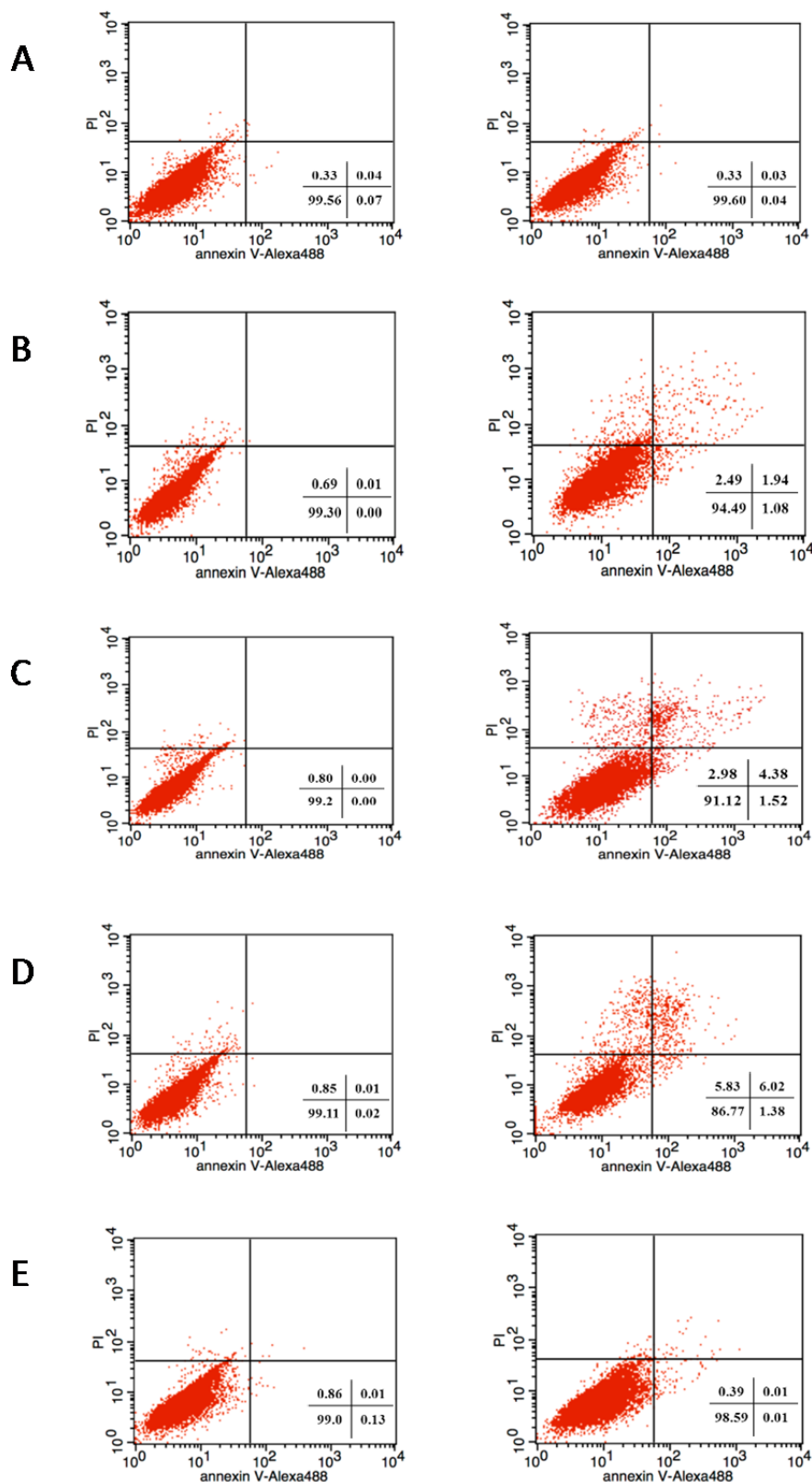


Figure 3: Effect of *P. falciparum* on bEnd.3 cells apoptosis upon cultivation with various percentages of hematocrit of Pf-IRBC for 4 (left panel) and 20 hours (right panel). Different conditions as (A) bEnd.3 cells control, (B) 1 % Hct, 50 % Pf-IRBC, (C) 3 % Hct, 50 % Pf-IRBC, (D) 5 % Hct, 50 % Pf-IRBC, (E) 5 % Hct, Uninfected RBC were tested. The apoptotic cells were analyzed by flow cytometry. These figures were representative of three independent experiments that yielded similar results. (AnV-/PI-: vital cell, AnV+/PI-: early apoptotic, AnV+/PI+: late apoptotic and AnV-/PI+: dead cell)

Table 1: Effect of parasitemia level and incubation period on apoptosis induction of bEnd.3 cells via *Plasmodium falciparum*-infected red blood cells (Pf-IRBC)

% Hematocrit ^a	% Apoptosis (range) ^b		With- ingroup ^c (P = 0.05)	Between- group ^d (P = 0.05)
	4 hours	20 hours		
1% Hct, 50% Pf-IRBC	0.05 ± 0.07	3.55 ± 0.29	P < 0.05	P < 0.05
3% Hct, 50% Pf-IRBC	0.06 ± 0.02	5.93 ± 0.15	P < 0.05	P < 0.05
5% Hct, 50% Pf-IRBC	0.09 ± 0.06	7.52 ± 0.11	P < 0.05	P < 0.05
5% Hct, Uninfected RBC	0.04 ± 0.02	0.03 ± 0.01	P > 0.05	NA

^a Various percentages of hematocrit of 50 % Pf-IRBC

^b Data represented as mean of values from three independent experiments ± SD

^c Data were compared within group (4 hours vs 20 hours)

^d Data were compared with control (Uninfected RBC) at 20 hours

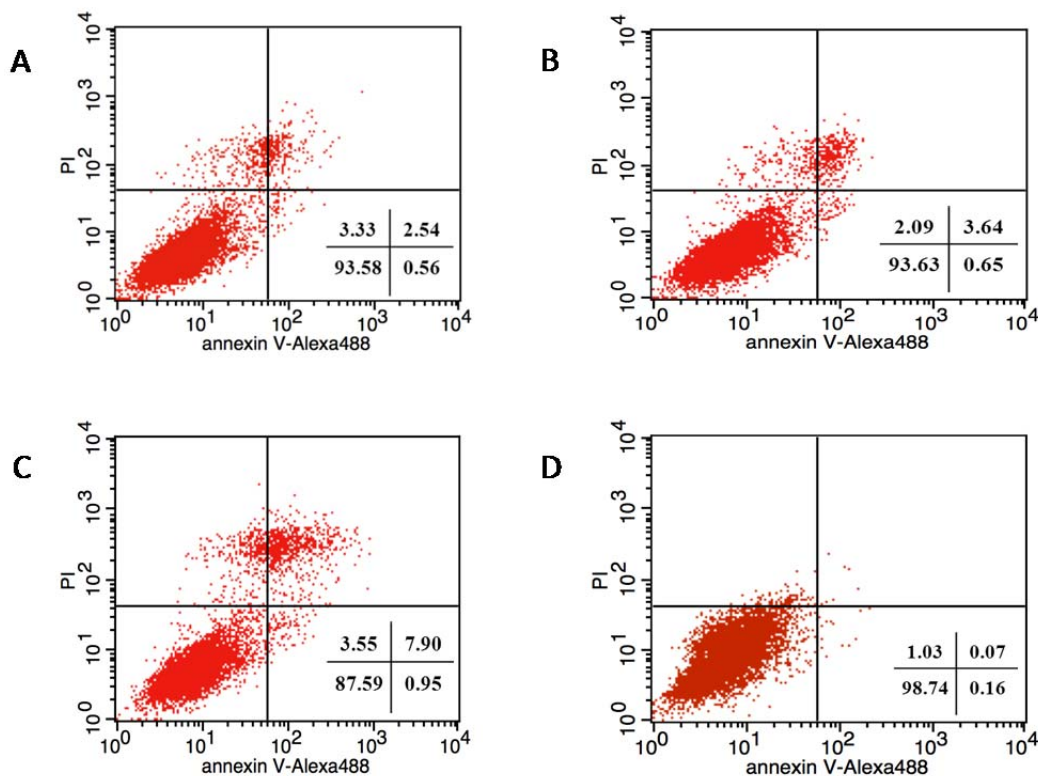


Figure 4: Effect of *Plasmodium falciparum*-infected red blood cells (Pf-IRBC), Platelets and PBMC on bEnd.3 cells apoptosis. Different conditions as (A) Pf-IRBC+Platelets, (B) Pf-IRBC+PBMC, (C) Pf-IRBC+Platelets+PBMC, (D) Uninfected RBC+Platelets+PBMC were tested. The bEnd.3 cells were cultured with Pf-IRBC, Platelets and PBMC at the condition of 1 % Hct, 50 % Pf-IRBC, 1×10^9 cells/cm² Platelets and 7.0×10^5 cells/cm² PBMC for 20 hours. These figures were representative of three independent experiments that yielded similar results (AnV-/PI-: vital cell, AnV+/PI-: early apoptotic, AnV+/PI+: late apoptotic and AnV-/PI+: dead cell).

Table 2: Influence of *Plasmodium falciparum*-infected red blood cells (Pf-IRBC) and influential factors (Platelets and PBMC) on bEnd.3 cells apoptosis

Influential factors ^a	% Apoptosis (range) ^b		Within group ^c (P = 0.05)	Between group ^d (P = 0.05)
	4 hours	20 hours		
Pf-IRBC	0.05 ± 0.07	3.55 ± 0.29	P < 0.05	P < 0.05
Pf-IRBC + Platelets	0.16 ± 0.01	3.32 ± 0.23	P < 0.05	P < 0.05
Pf-IRBC + PBMC	0.17 ± 0.15	4.61 ± 0.12	P < 0.05	P < 0.05
Pf-IRBC + Platelets + PBMC	0.51 ± 0.05	10.77 ± 0.54	P < 0.05	P < 0.05
Uninfected RBC + Platelets + PBMC	0.13 ± 0.02	0.27 ± 0.30	P > 0.05	NA

^a Pf-IRBC, PBMC and Platelets were used at 1 % Hct, 50 % Pf-IRBC, 7.0×10^5 cells/cm² and 1×10^9 cells/cm², respectively

^b Data represent as mean of values from three independent experiments ± SD

^c Data were compared within group (4 hours vs 20 hours)

^d Data were compared with control (Uninfected RBC + Platelets + PBMC) at 20 hours

function. Then, therapeutics that can modulate key endothelial functions need to be tested as an adjunctive treatment in severe malaria and CM.

Here, this study successfully explored the effect of curcumin on parasite elimination and endothelial protection in vitro systems. We found that curcumin inhibited the growth of *P. falciparum* in a dose-dependent fashion; with an IC₅₀ of ~10 μM. Our study gave a result similar to other studies (Chakrabarti et al., 2013; Nandakumar et al., 2006; Reddy et al., 2005). Moreover, curcumin did not show any cytotoxic effects when applied to cultured bEnd.3 cells for a long period of time.

Role of Pf-IRBC on brain endothelial cells apoptosis was investigated. Our findings showed that Pf-IRBC induced bEnd.3 cells apoptosis, which is amplified by parasitemia levels and incubation period (Table 1). However, the mechanism of brain endothelial cells apoptosis triggered by Pf-IRBC is still unclear. Recently, Pino and co-workers (2003) reported that Pf-IRBC cytoadherence could modulate brain endothelial expression of TNF-α superfamily genes and apoptosis-related genes (Bad, Bax, caspases and iNOS). In addition, Pf-IRBC adhesion to brain endothelial directly activated the Rho kinase signaling pathway and

induced the production of reactive oxygen species (ROS) by endothelial cells, both pathways could potentially lead to cell death (Pino et al., 2003).

However, a number of other mechanisms has been proposed and Pf-IRBC might not be the only factor determining endothelial dysfunction during infection (Gitau and Newton, 2005). Several possible mechanisms can also cause endothelial dysfunction. The first is specific cell adhesion events (sequestration) and second includes adhesion-independent pathways, which may or may not require the presence of sequestered parasites within the vessel.

Such events might activate secondary signaling cascades and could induce toxic local microvascular environment such as hypoxia or metabolic competition. The release of soluble parasite products acts as toxins or autocrine for the regulation of endothelial function due to cytokine release (Hunt and Grau, 2003) or other mediators released by host leukocytes. The supportive reason for this mechanism had been studied in the brain of CM cases. Transmigration of host leukocytes and over-expression of pro-inflammatory cytokines tumor necrosis factor (TNF)-α, IL-1β and transforming growth factor (TGF-β) were observed in post-mortem studied (Brown et al., 1999).

Our study supported the role of host response on endothelial dysfunction during malarial infection. We showed that increasing of bEnd.3 cells apoptosis significantly depended on a synergistic effect between parasitemia and host cells; platelets and PBMC (Table 2).

Undoubtedly, brain endothelial cells represent as a key interface between the *P. falciparum* and the human host during malarial infection. Thus, a strategy combining parasite elimination and endothelial protection is of great interest. Our study showed the capacity of curcumin that possessed both antimalarial activity and endothelial protection upon cultivation with Pf-IRBC, platelets and PBMC in both pre-treatment and co-treatment in vitro (Table 3).

In context of malaria, curcumin has been shown to possess moderate antimalarial activity with IC₅₀ value of 5–18 μM (Chakrabarti et al., 2013; Nandakumar et al., 2006; Reddy et al., 2005). Curcumin not only acts itself antimalarial activity but also implicates in many targets in CM as follows:

- i. Curcumin modulates NF-κB (nuclear factor κ beta), which plays a vital role in malaria. Pf-IRBC has been shown to induce NF-κB regulated inflammatory pathway in human cerebral endothelium. Antimalarial activity in curcumin against the blood phase of the life cycle may lead to its role in regulation of NF-κB. Curcumin has been evidenced to reduce the production of proinflammatory cytokines such as TNF, IL-12 and IL-6 in vitro (Golenser et al., 2006). It can be concluded that curcumin can effectively control inflammatory cascade as a result of host immune response in CM.
- ii. Curcumin inhibits expression of various cell surface adhesion molecules such as ICAM-1, VCAM-1 and endothelial leukocyte adhesion molecule (ELAM-1) on endothelial cell (Kunnumakkara et al., 2008). Curcumin inhibits adhesion of platelets to brain endothelial cells in

vitro, which accumulates in brain vessels in CM patients (Zhang et al., 2008). These data support the development of adjunctive therapies to reverse the pathophysiological consequences of cytoadherence.

- iii. Curcumin inhibits histone acetyltransferase (HAT) that causes parasite chromatin modifications and has antiparasitic effects (Cui and Miao, 2007).
- iv. Curcumin inhibits iNOS by suppression of IFN-γ and IL-12 production. iNOS has been shown to mediate production of ROS (Pan et al., 2000).
- v. Curcumin upregulates heme oxygenase-1 (HO-1) gene and protein expression by protecting the brain endothelial cells from peroxide mediated toxicity as well as toxicity caused due to free heme. (Mottlerlini et al., 2000).
- vi. Curcumin suppresses C-Jun N terminal kinases (JNK) activation. JNK belongs to the family of mitogen activated kinases (MAP kinases), which are activated in response to inflammatory cytokines and stress conditions. Its activation induces the transcription-dependent apoptotic signalling pathway, resulting in cell death during experimental CM (Anand and Babu, 2011).
- vii. In a murine model of CM, the administration of curcumin results in partial alleviation of CM and delayed death (Waknine-Grinberg et al., 2010).

Taken altogether, curcumin has been evidenced to possess both antimalarial activity and endothelial protection. This can be proposed as an ideal antimalarial compound especially for use in combination with antimalarial drugs such as artesunate not only to limit the use but also to overcome the problems of high cost and drug resistance. In view of its abundance, non-toxic nature compound, and exhibited therapeutic effects in a variety of human diseases, it will be useful to further investigate the feasibility of applying curcumin as low-cost adjunctive antimalarial agents.

Table 3: Protection/neutralization effects of curcumin on bEnd.3 cells apoptosis upon cultivation with Pf-IRBC, Platelets and PBMC at 20 hours

Treatment agents ^a	% Apoptosis (range) ^b		% Apoptosis reduction ^c	
	Pre-Treatment	Co-Treatment	Pre-Treatment	Co-Treatment
Curcumin	4.16 ± 0.16	2.12 ± 0.15	60.0	79.6
Tocopherol	6.32 ± 0.16	7.68 ± 0.37	39.4	26.2
Artesunate	1.93 ± 0.08	0.41 ± 0.03	81.4	96.1

^a Flour treatment agents: Curcumin (10 µM), Tocopherol (10 µM), Artesunate (10 nM)

^b Data were mean of values from three independent experiments ± SD

^c Data were compared with control experiment: Pf-IRBC + Platelets + PBMC (bEnd.3 cell apoptosis 10.4 ± 0.73 %)

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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