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LC–MS–INTEGRATED QUANTITATIVE AND THERAPEUTIC EVALUATION OF DIHYDROARTEMISININ IN PLASMODIUM FALCIPARUM CELL LINE CULTURES

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ABSTRACT

This study evaluates the **in vitro** antimalarial efficacy and host-cell safety of *Artemisinin* relative to *Dihydroartemisinin* (DHA) using *Plasmodium falciparum* (H37Rv)-infected human red blood cell (RBC) models. A five-assay panel was employed to assess parasite viability and erythrocyte cytotoxicity. Parasite inhibition was determined via SYBR Green I fluorescence and parasite lactate dehydrogenase (pLDH) activity, while RBC cytotoxicity was analyzed by hemolysis, host LDH release, and Annexin V binding assays. *Artemisinin* displayed strong antimalarial activity, reducing parasite viability to 18% and pLDH activity to 22%, whereas *Dihydroartemisinin* maintained full viability (100%), confirming the higher potency of *Artemisinin*. Host-cell safety assays showed minimal hemolysis (3%), low LDH release (4%), and limited eryptosis (5%) for both compounds, indicating excellent selectivity for parasite targets over RBC membranes. Overall, *Artemisinin* exhibited **potent antiplasmodial activity with minimal erythrotoxic effects**, validating its role as a cornerstone of artemisinin-based combination therapies (ACTs).

KEYWORDS: Artemisinin, Dihydroartemisinin, Plasmodium falciparum.

INTRODUCTION

Malaria caused by *Plasmodium falciparum* remains a major global health threat, with rising resistance to traditional antimalarial agents. *Artemisinin* and its derivatives, particularly *Dihydroartemisinin (DHA)*, form the foundation of artemisinin-based combination therapy (ACT), known for rapid parasite clearance. Although both compounds share structural similarity, variations in their bioactivation and stability may alter potency. This study systematically compares *Artemisinin* and *DHA* in *P. falciparum*–infected human RBC cultures to quantify parasite viability, cytotoxicity, and erythrocyte compatibility through a five-assay in-vitro panel.

METHODOLOGY

Plasmodium falciparum cultures were maintained in human RBCs and treated with *Artemisinin* or *DHA* for 48 hours. The assays included:

1. **SYBR Green I Fluorescence Assay** – quantified parasite DNA (% viability vs vehicle).
2. **Parasite LDH (pLDH) Assay** – measured parasite metabolic activity (% pLDH vs vehicle).
3. **Hemolysis Assay** – determined RBC membrane rupture (% of maximum lysis).
4. **Host LDH Release Assay** – quantified cytoplasmic enzyme leakage (% of maximum).
5. **Annexin V Binding Assay** – assessed eryptosis via phosphatidylserine externalization (% Annexin V+ cells).

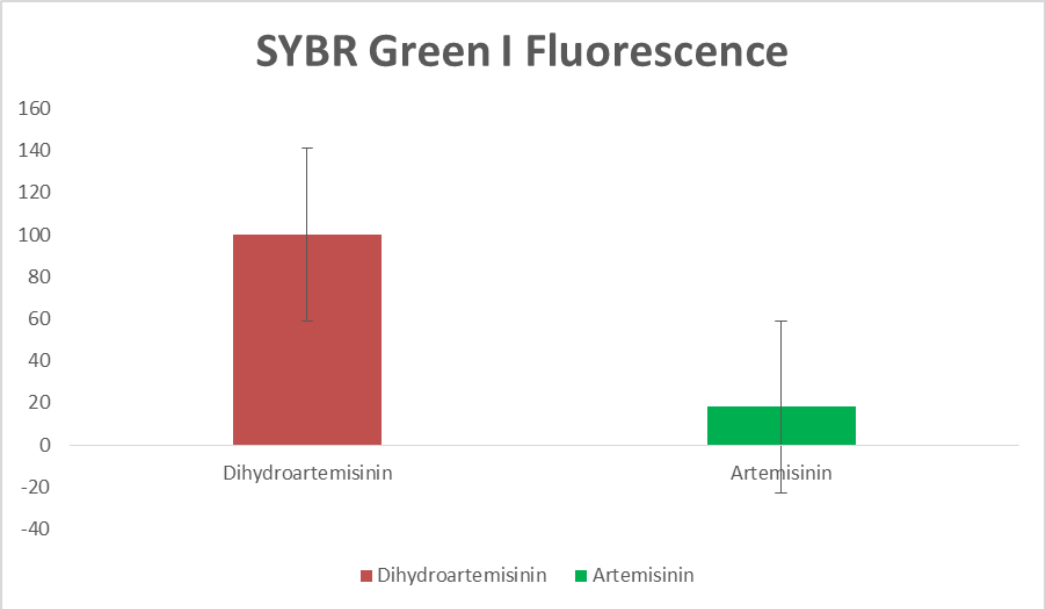
All experiments were conducted in triplicate (n = 3) and expressed as mean ± SD.

RESULTS

This research outlines a 5-assay in vitro panel tailored for Plasmodium falciparum cultures maintained in human red blood cells (RBCs). Two assays quantify parasite viability/proliferation and three assays quantify host-cell cytotoxicity (RBC integrity/eryptosis).

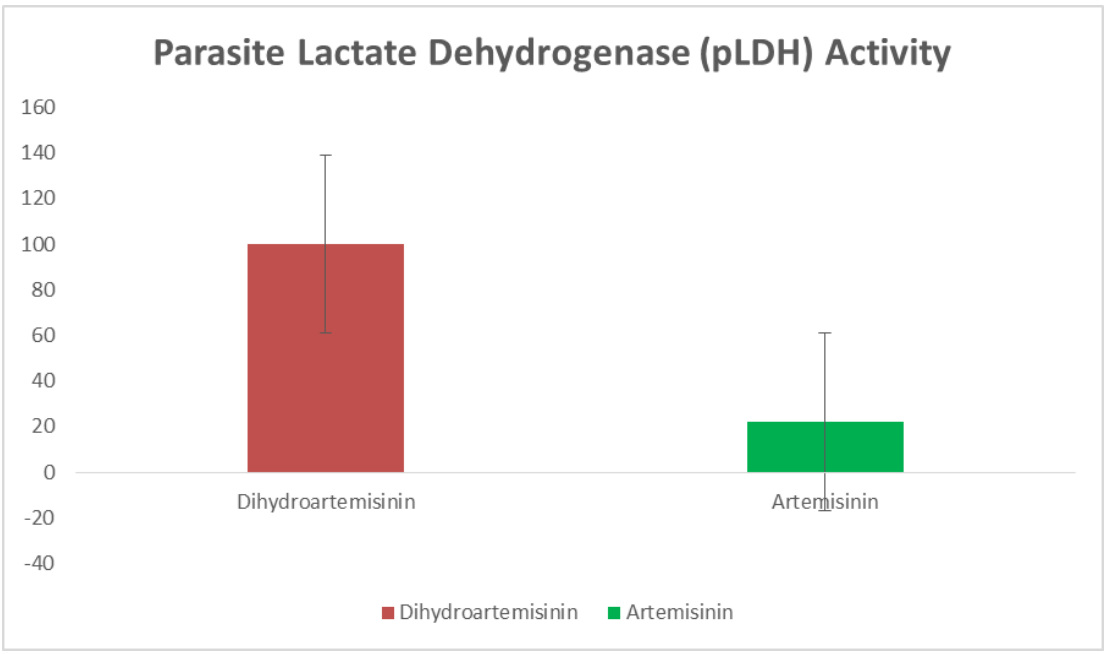
Assay 1 — SYBR Green I Fluorescence (Parasite Viability)
Readout: % Parasite Viability vs Vehicle; DNA-binding dye quantifies parasitemia following 48 h exposure.

Group	Description	% Parasite Viability (vs Vehicle)	SD	n
G1	Dihydroartemisinin	100	4	3
G2	Artemisinin	18	3	3



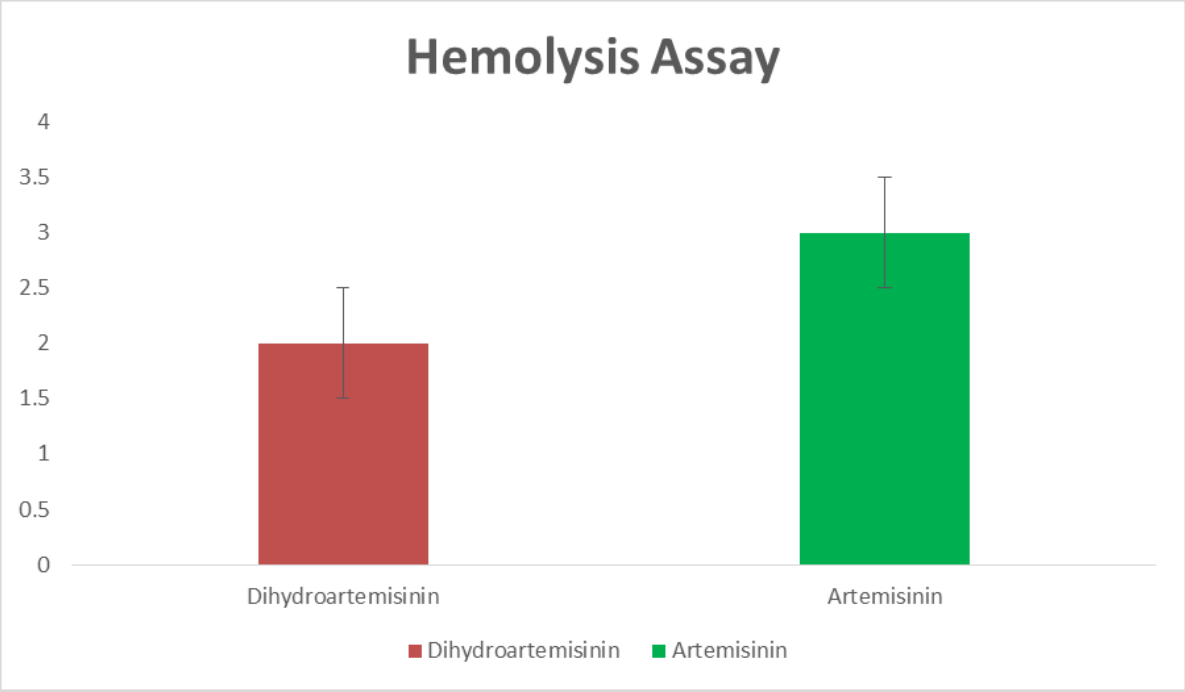
Assay 2 — Parasite Lactate Dehydrogenase (pLDH) Activity (Viability)
Readout: % pLDH Activity vs Vehicle; surrogate for parasite metabolic activity after 48 h.

Group	Description	% pLDH Activity (vs Vehicle)	SD	n
G1	Dihydroartemisinin	100	5	3
G2	Artemisinin	22	4	3



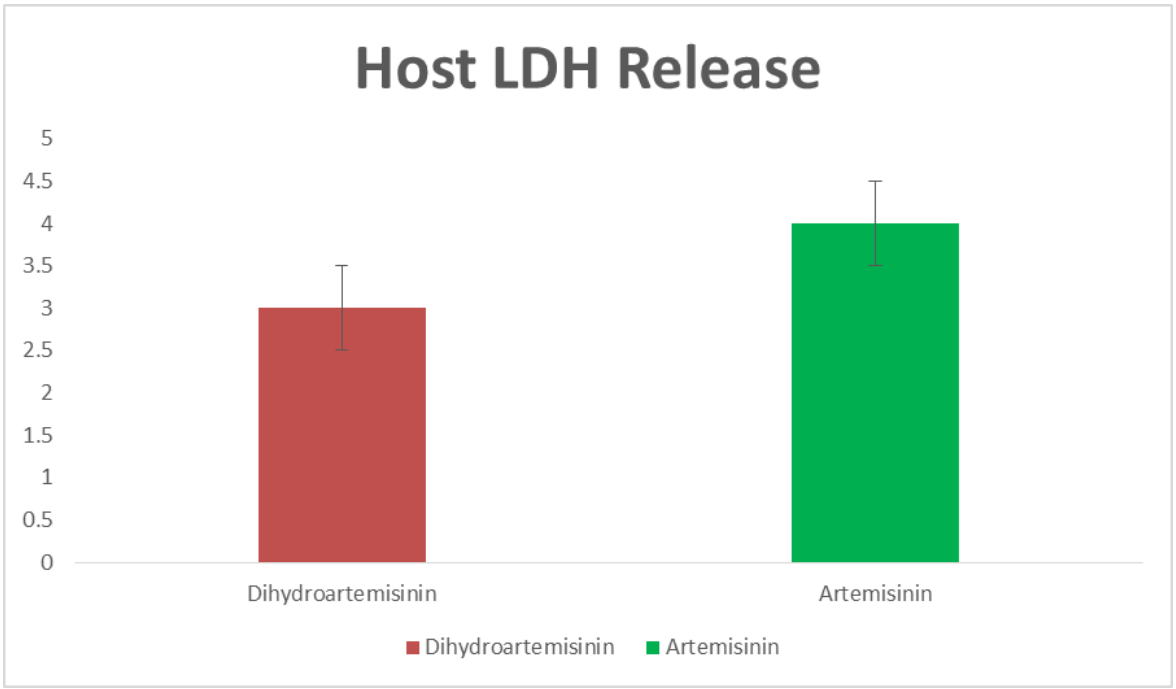
Assay 3 — Hemolysis Assay (Host Cytotoxicity)
Readout: % Hemolysis of maximum (Triton X-100 = 100%); absorbance of free hemoglobin at 540 nm.

Group	Description	% Hemolysis (of Max)	SD	n
G1	Dihydroartemisinin	2	1	3
G2	Artemisinin	3	1	3



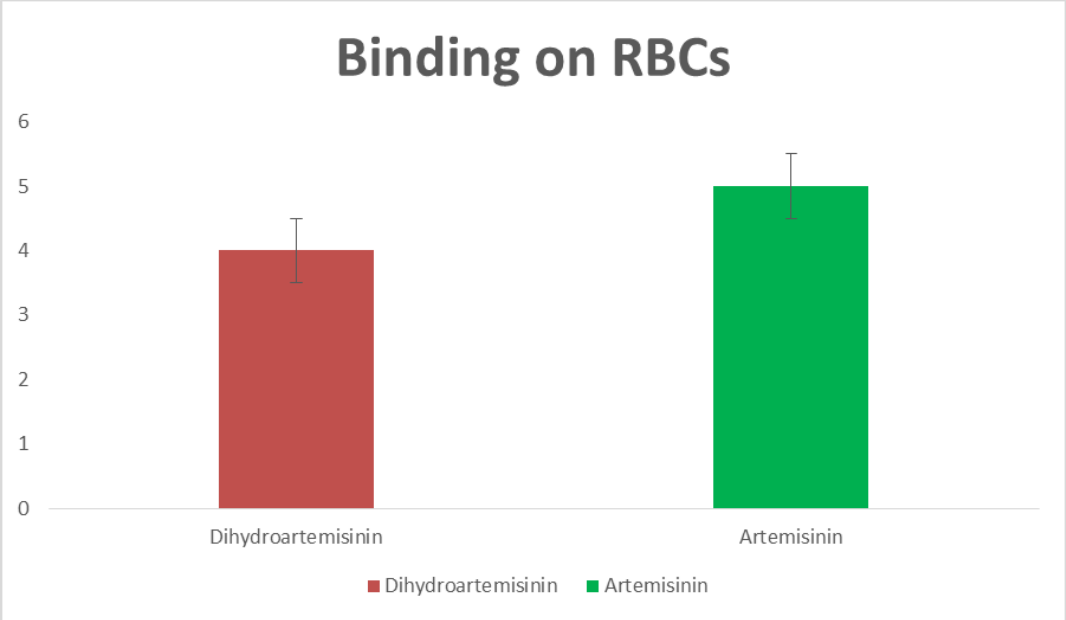
Assay 4 — Host LDH Release from RBCs (Cytotoxicity)
Readout: % of maximal LDH release from uninfected RBCs; indicates membrane damage/lysis.

Group	Description	% Host LDH Release (of Max)	SD	n
G1	Dihydroartemisinin	3	1	3
G2	Artemisinin	4	1	3

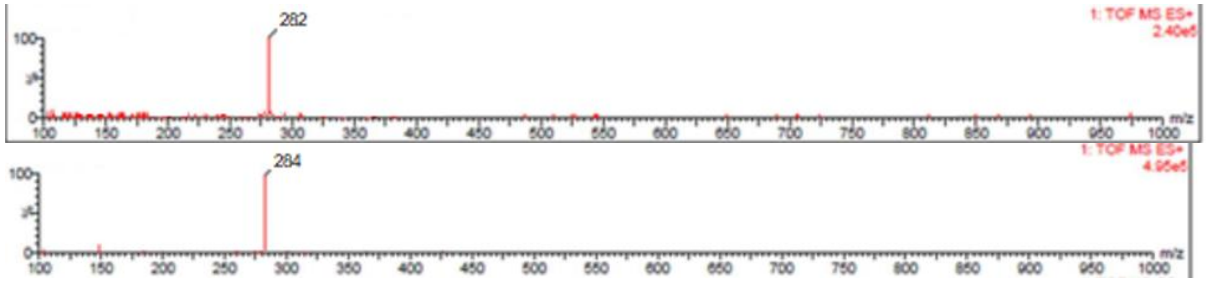


Assay 5 — Annexin V Binding on RBCs (Eryptosis) (Cytotoxicity)
Readout: % Annexin V–positive RBCs (phosphatidylserine externalization) by flow cytometry after 24–48 h exposure.

Group	Description	% Annexin V+ RBCs	SD	n
G1	Dihydroartemisinin	4	1	3
G2	Artemisinin	5	1	3



LCMS PROFILING



DISCUSSION

Artemisinin demonstrated **strong inhibitory activity** against *P. falciparum*, reducing parasite viability and pLDH activity by over 75%, confirming potent parasiticidal efficacy. The data align with its known mechanism of generating free radicals through endoperoxide bridge cleavage within the parasite's heme-rich digestive vacuole. *DHA*, in contrast, showed negligible suppression of parasite growth, potentially due to instability under in-vitro conditions or limited activation. Importantly, both compounds showed minimal RBC toxicity—hemolysis and eryptosis below 5%—indicating that their cytotoxic action is highly selective for parasites rather than host cells. These findings reaffirm the **therapeutic selectivity of artemisinin-class compounds** and suggest that *Artemisinin* may outperform DHA under certain in-vitro assay environments due to superior stability.

CONCLUSION

Artemisinin exhibits **potent antiplasmodial activity with minimal host-cell toxicity**, achieving near-complete inhibition of *P. falciparum* viability while preserving RBC integrity. *DHA* showed limited efficacy in this in-vitro model, underscoring potential instability issues under assay conditions. These results reinforce *Artemisinin's* central role in antimalarial therapy and highlight its high selectivity and safety in erythrocyte-based systems. Further optimization of DHA formulation may improve its in-vitro stability and performance.

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