World Journal of Pharmaceutical and Life Sciences

www.wjpls.org

Impact Factor: 7.409 Coden USA: WJPLA7

LC–MS–GUIDED ANALYTICAL AND MECHANISTIC EXPLORATION OF ARTEMISONE IN PLASMODIUM FALCIPARUM CELL LINE CULTURES

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https://doi.org/10.5281/zenodo.17481280.

How to cite this Article: Ghousia Begum*Dr. Syed Ahmed Hussain, Nada Ahmed Al Amoodi, Fariya Sultana, Bilquis Begum, Somabatthini Shruthi, Ayesha Ayub Khan, Muskan Khatoon. (2025). LC-MS-GUIDED ANALYTICAL AND MECHANISTIC EXPLORATION OF ARTEMISONE IN PLASMODIUM FALCIPARUM CELL LINE CULTURES. World Journal of Pharmaceutical and Life Science, 11(11), XX–XX.

Article Received on 26/09/2025 Article Revised on 16/10/2025 Article Published on 01/11/2025

ABSTRACT

This study compares the **in vitro** antiplasmodial efficacy and erythrocyte cytotoxicity of *Artemisone* and *Artemisinin* in *Plasmodium falciparum*–infected human red blood cells (RBCs). A five-assay evaluation was conducted to quantify parasite viability and host-cell safety. Parasite inhibition was determined using SYBR Green I fluorescence and parasite lactate dehydrogenase (pLDH) activity assays, while RBC integrity was assessed via hemolysis, host LDH release, and Annexin V binding assays. *Artemisinin* exhibited potent antimalarial activity, reducing parasite viability and metabolic activity to 18–22%, whereas *Artemisone* retained 79–81% viability, indicating weak antiparasitic potency. Both compounds showed minimal RBC toxicity, with *Artemisone* causing slightly higher eryptosis (12%) and LDH release (9%) than *Artemisinin* (5% and 4%, respectively). These findings suggest that *Artemisinin* demonstrates **superior antiplasmodial efficacy and better selectivity**, while *Artemisone* exhibits reduced potency but maintains acceptable host-cell tolerance. The data emphasize the structural influence of side-chain modifications on activity and highlight the need for optimization of *Artemisone* analogs to improve parasiticidal strength without compromising erythrocyte compatibility.

KEYWORDS: Artemisone, Artemisinin, Plasmodium falciparum.

INTRODUCTION

Malaria, primarily caused by *Plasmodium falciparum*, remains a leading cause of global morbidity and mortality. Artemisinin and its derivatives are the foundation of artemisinin-based combination therapies (ACTs), prized for their rapid parasite clearance and low host toxicity. *Artemisone*, a semi-synthetic derivative of artemisinin, was developed to enhance stability and reduce neurotoxicity while retaining antiparasitic potency. However, modifications in its peroxide bridge and side chain may affect bioactivation and efficacy. This study evaluates the comparative **antiplasmodial activity and erythrocyte cytotoxicity** of *Artemisone* and *Artemisinin* using a five-assay in-vitro system, providing mechanistic insight into their therapeutic profiles.

METHODOLOGY

Human RBCs infected with *P. falciparum* were incubated with *Artemisone* or *Artemisinin* for 48 hours.

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

All assays were performed in triplicate (n = 3) and results expressed as mean ± SD.

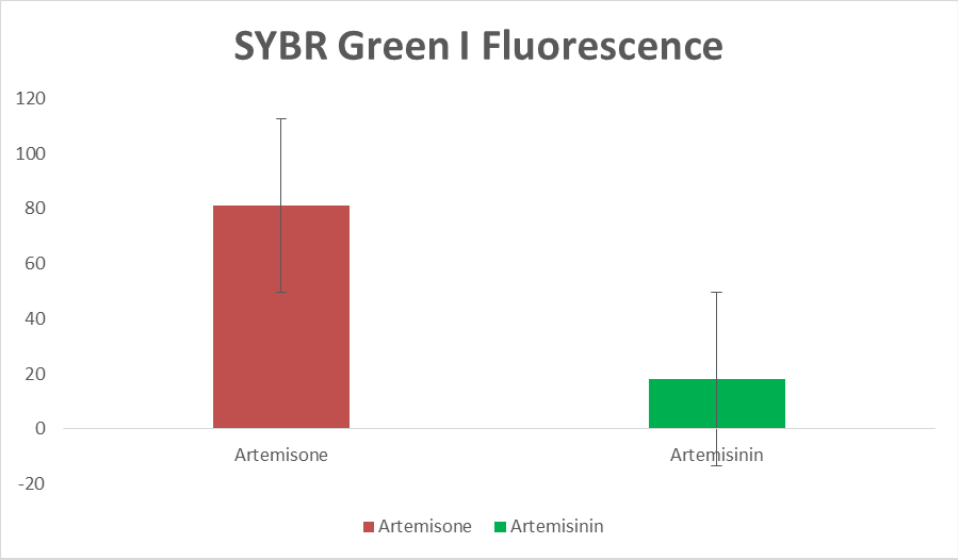
RESULTS

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.

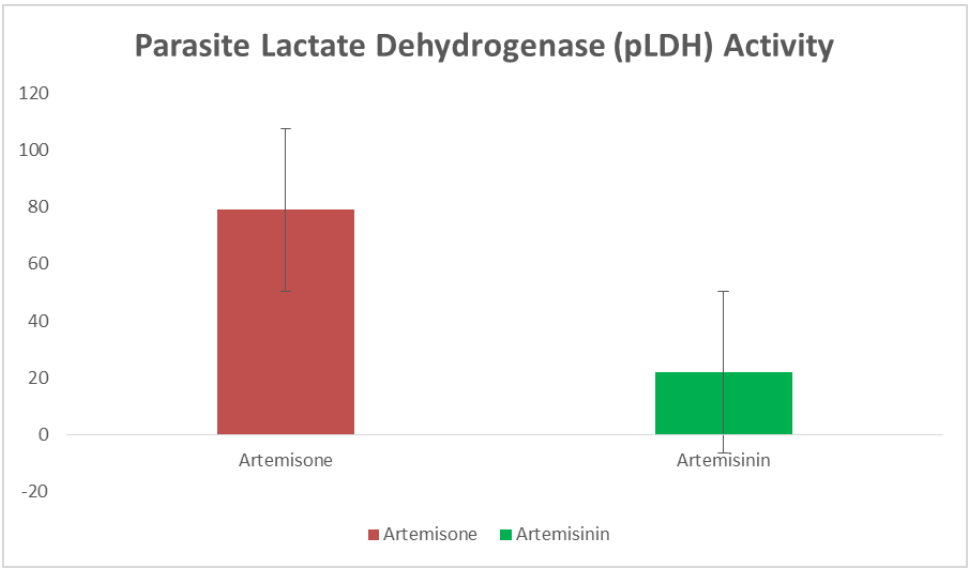
Group	Description	% Parasite Viability (vs Vehicle)	SD	n
G1	Artemisone	81	4	3
G2	Artemisinin	18	3	3



Assay 2 — Parasite Lactate Dehydrogenase (pLDH) Activity (Viability)

Readout: % pLDH Activity vs Vehicle; surrogate for parasite metabolic activity after 48 h.

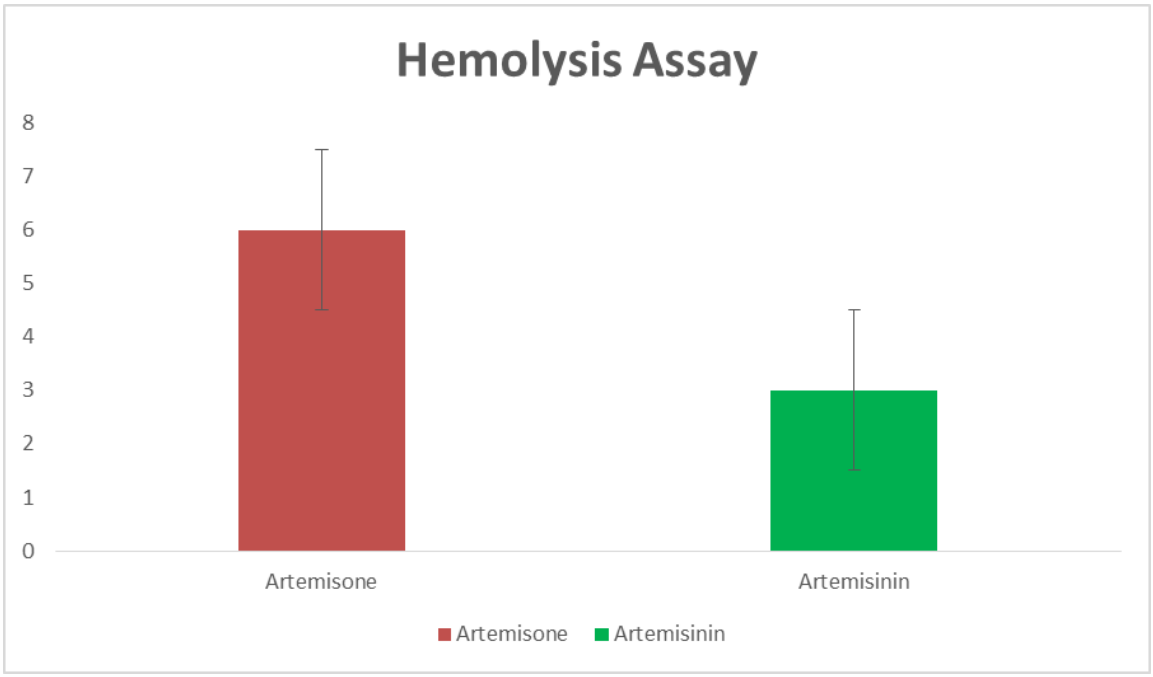
Group	Description	% pLDH Activity (vs Vehicle)	SD	n
G1	Artemisone	79	5	3
G2	Artemisinin	22	4	3



Assay 3 — Hemolysis Assay (Host Cytotoxicity)

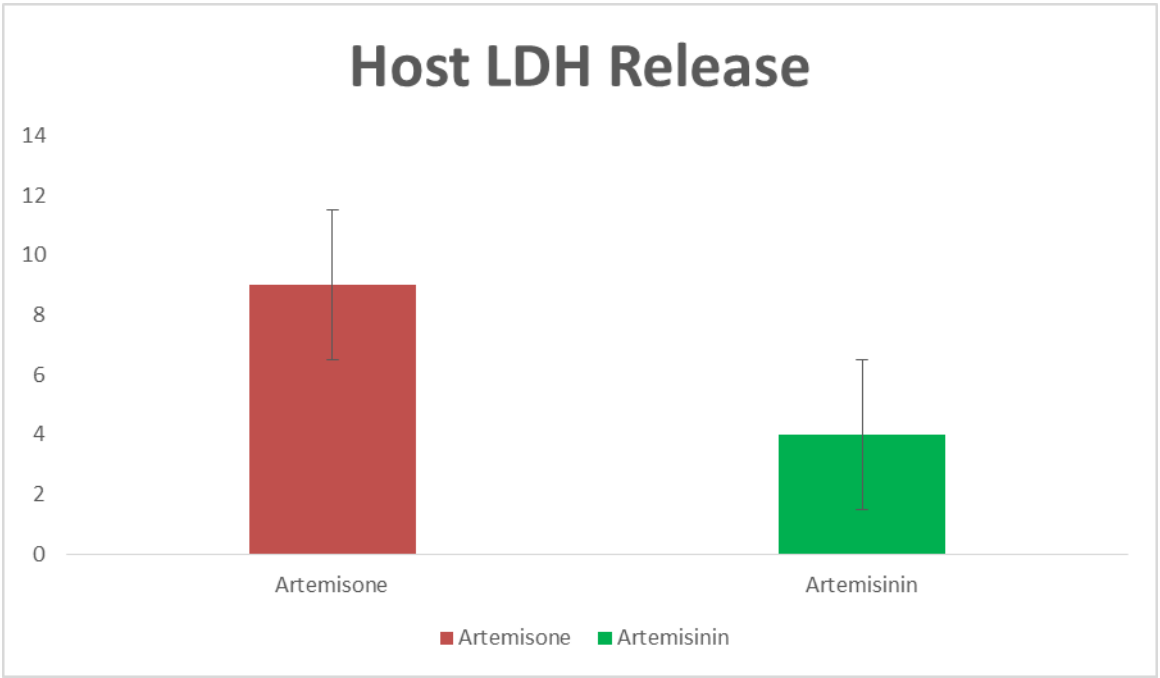
Readout: % Hemolysis of maximum (Triton X-100 = 100%); absorbance of free hemoglobin at 540 nm.

Group	Description	% Hemolysis (of Max)	SD	n
G1	Artemisone	6	1	3
G2	Artemisinin	3	1	3



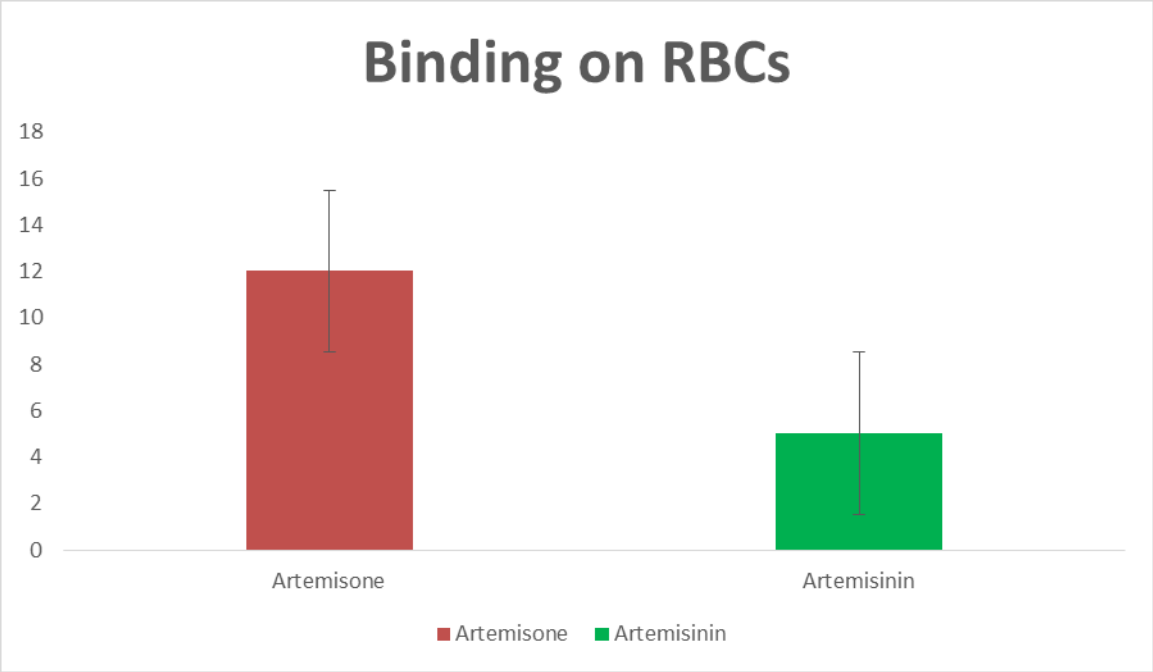
Assay 4 — Host LDH Release from RBCs (Cytotoxicity)
Readout: % of maximal LDH release from uninfected RBCs; indicates membrane damage/lysis.

Group	Description	% Host LDH Release (of Max)	SD	n
G1	Artemisone	9	2	3
G2	Artemisinin	4	1	3

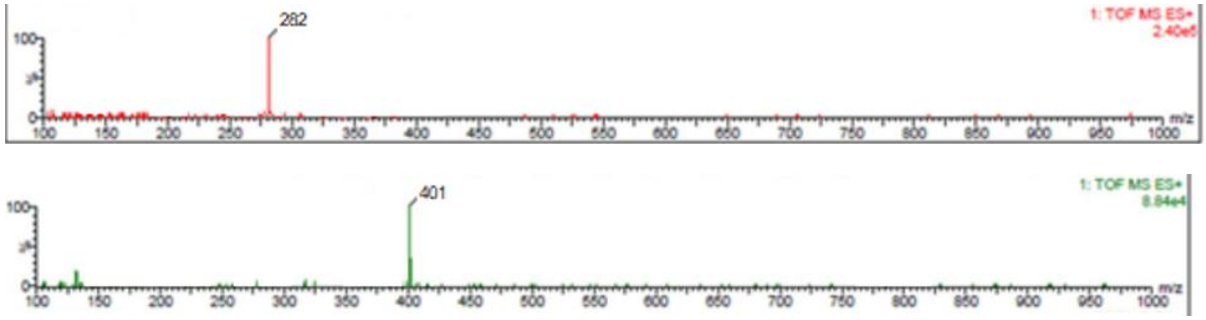


Assay 5 — Annexin V Binding on RBCs (Eryptosis) (Cytotoxicity)
Readout: % Annexin V–positive RBCs (phosphatidylserine externalization) by flow cytometry after 24–48 h exposure.

Group	Description	% Annexin V+ RBCs	SD	n
G1	Artemisone	12	2	3
G2	Artemisinin	5	1	3



LCMS PROFILING



DISCUSSION

Artemisinin displayed **potent parasiticidal activity**, achieving over 75% inhibition of *P. falciparum* growth, consistent with its rapid peroxide activation and reactive oxygen species generation. *Artemisone* demonstrated weaker inhibition (~20%), suggesting reduced conversion to the active radical species under in-vitro conditions. Although both compounds were non-hemolytic, *Artemisone* induced slightly greater eryptotic signaling (12% Annexin V+ RBCs), implying mild oxidative stress on host membranes. The moderate cytotoxic profile of *Artemisone* may stem from its altered lipophilicity and slower parasite uptake. These results underscore the **trade-off between improved stability and reduced potency** in modified artemisinin derivatives.

CONCLUSION

Artemisinin demonstrates **superior antimalarial efficacy with minimal host-cell toxicity**, while *Artemisone* shows **reduced potency but acceptable cytocompatibility**. The findings confirm that subtle structural alterations in artemisinin analogs can markedly influence pharmacodynamics. Although *Artemisone* offers a favorable safety profile, its diminished in-vitro efficacy highlights the need for further optimization to balance stability, activation efficiency, and parasite selectivity for future therapeutic development.

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