



## Dose–Timing Effects of Ascorbic Acid on Dihydroartemisinin Efficacy

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### ABSTRACT

Artemisinin-based therapies remain the primary treatment for malaria; however, the concurrent use of antioxidant supplements during therapy is common in endemic regions. Emerging evidence suggests that antioxidants such as ascorbic acid (AA) may interfere with the antimalarial efficacy of dihydroartemisinin (DHA). The present study investigates the dose- and timing-dependent interaction between AA and DHA using a *Plasmodium berghei*-infected murine model. Multiple AA dosing regimens and administration schedules were evaluated to assess parasite clearance, oxidative stress, and hepatic injury markers. The results demonstrate that high-dose and simultaneous AA administration significantly attenuates DHA-mediated parasite clearance, whereas delayed AA administration preserves antimalarial efficacy while partially modulating hepatic oxidative stress. These findings highlight the importance of dosing strategy and timing in minimizing adverse drug–nutrient interactions and support the development of evidence-based guidelines for antioxidant supplementation during malaria therapy.

**KEY WORDS:** Dihydroartemisinin; Ascorbic Acid; Dose–Timing Interaction; Malaria; Oxidative Stress; Drug–Nutrient Interaction

## Introduction

Malaria continues to pose a significant global health burden, particularly in low- and middle-income countries where access to healthcare resources remains limited [2]. Artemisinin-based combination therapies (ACTs) are the cornerstone of malaria treatment due to their rapid parasitocidal activity and favorable safety profile. Dihydroartemisinin (DHA), the active metabolite of several artemisinin derivatives, plays a central role in these regimens by inducing iron-dependent oxidative damage within malaria parasites [2].

In parallel with antimalarial therapy, the use of vitamin and antioxidant supplements is widespread in malaria-endemic regions. Ascorbic acid (vitamin C) is commonly administered to improve immune function, counteract oxidative stress, and manage anemia associated with infection. While antioxidants are generally perceived as beneficial, their concurrent use with redox-dependent drugs such as artemisinin derivatives has raised concerns regarding potential antagonistic interactions [3, 4].

Recent experimental studies suggest that AA may reduce the efficacy of DHA by suppressing oxidative stress and interfering with iron-mediated radical formation. However, limited attention has been given to the influence of AA dose and timing relative to DHA administration. Understanding whether these interactions can be mitigated through optimized dosing schedules is critical for improving therapeutic outcomes [5].

This study aims to evaluate the dose- and timing-dependent effects of AA on DHA antimalarial efficacy and hepatic

oxidative responses, providing translational insight into safer supplementation strategies during malaria treatment.

## Experimental Design and Methodology

### Experimental Animals and Ethical Considerations

Male Swiss albino mice aged 6–8 weeks and weighing 25–30 g were used in this study. Animals were housed under standard laboratory conditions, including controlled temperature (22–25°C), a 12 h light/dark cycle, and unrestricted access to standard pellet diet and drinking water. All experimental procedures were conducted in accordance with internationally accepted guidelines for the care and use of laboratory animals and were approved by the institutional animal ethics committee.

### Parasite Strain and Inoculation

Experimental malaria was induced using the rodent malaria parasite *Plasmodium berghei*. Donor mice with established parasitemia were used to prepare infected erythrocyte suspensions. Each experimental mouse was inoculated intraperitoneally with a standardized inoculum of *P. berghei*-infected red blood cells diluted in sterile physiological saline. Successful parasite establishment was confirmed by microscopic examination of Giemsa-stained blood smears prior to initiation of treatment.

### Dose- and Timing-Dependent Treatment Design

To investigate the influence of both dose and timing of ascorbic acid (AA) on dihydroartemisinin (DHA) efficacy, animals were randomly assigned into six experimental groups

(n = 4 per group):

- Infected untreated group
- DHA alone (5 mg/kg)
- DHA + AA (low dose: 2 mg/kg, administered simultaneously)
- DHA + AA (high dose: 10 mg/kg, administered simultaneously)
- DHA followed by AA (5 mg/kg, AA administered 12 h after DHA)
- AA followed by DHA (5 mg/kg, DHA administered 12 h after AA)

All treatments were administered orally using a gastric gavage once daily for four consecutive days. The selected doses and time intervals were based on previous pharmacological studies and were designed to reflect clinically relevant supplementation practices.

### Monitoring of Parasitemia

Parasitemia was monitored daily throughout the treatment period. Thin blood smears were prepared from tail vein blood, air-dried, fixed with methanol, and stained using Giemsa stain. Stained smears were examined under a light microscope, and parasitemia was calculated as the percentage of infected erythrocytes among at least 1,000 counted cells.

### Assessment of Oxidative Stress and Liver Function

At the end of the treatment period, animals were sacrificed and liver tissues were excised, rinsed in ice-cold saline, and homogenized under cold conditions. Hepatic homogenates were used for the determination of reactive oxygen species (ROS), malondialdehyde (MDA) levels, and antioxidant enzyme activities. Blood samples were collected for serum separation, and alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured using standard diagnostic kits to assess hepatic injury [7].

### Statistical Analysis

All experimental data were expressed as mean  $\pm$  standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by appropriate post hoc comparisons. A probability value of  $p < 0.05$  was considered statistically significant.

## Dose–Timing Dependent Effects of Ascorbic Acid on DHA Therapy

### Effect of Ascorbic Acid Dose on Parasite Clearance

DHA monotherapy resulted in significant parasite suppression. Low-dose AA co-administration produced a modest reduction in efficacy, whereas high-dose AA significantly impaired parasite clearance. These findings indicate a clear dose-dependent antagonistic effect of AA on DHA activity.

### Timing of Administration Influences Therapeutic Outcome

Simultaneous administration of DHA and AA resulted in the greatest reduction in antimalarial efficacy. In contrast, delayed administration of AA following DHA preserved parasite clearance while moderating hepatic oxidative stress. Conversely, AA pre-treatment reduced DHA effectiveness.

## Hepatic Oxidative Stress and Liver Enzyme Response

High-dose and simultaneous AA administration significantly reduced oxidative stress markers but was associated with suboptimal parasite clearance. Delayed AA dosing achieved a balance between oxidative stress modulation and therapeutic efficacy, as reflected by moderate ALT and AST levels.

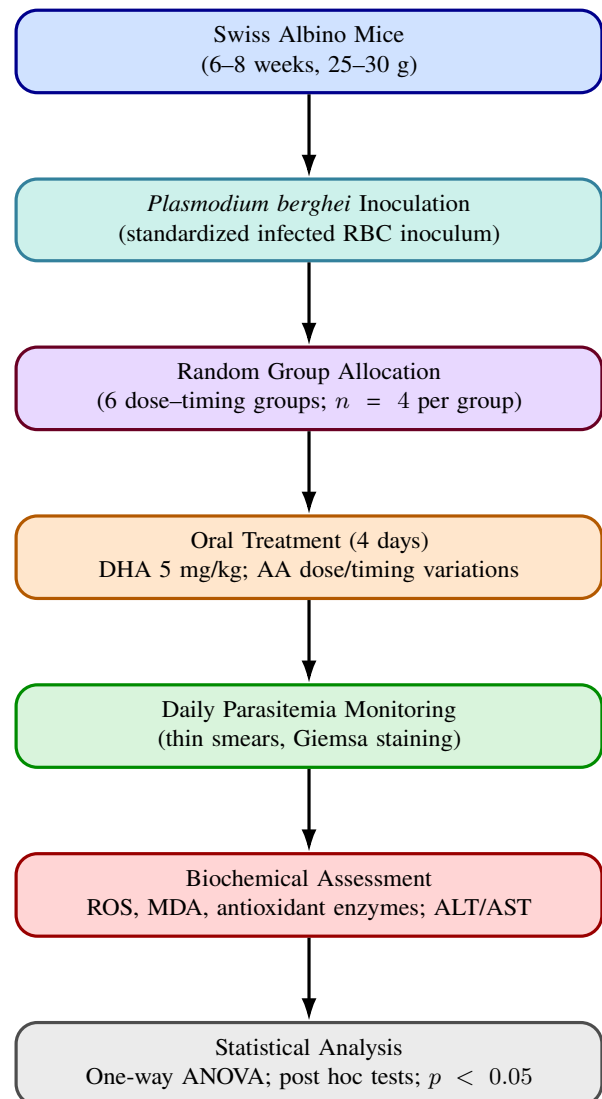


Figure 1: Colored workflow of the dose–timing experimental design evaluating the interaction between ascorbic acid and dihydroartemisinin in *P. berghei*-infected mice.

## Translational Implications of Dose and Timing Optimization

The present study demonstrates that the interaction between ascorbic acid (AA) and dihydroartemisinin (DHA) is strongly dependent on both the *dose* of AA and the *timing* of its administration relative to DHA. Simultaneous co-administration, particularly at higher AA dose levels, produced the most pronounced attenuation of DHA-mediated parasite clearance. This pattern supports a clinically important interpretation: excessive antioxidant buffering can reduce the redox-dependent pharmacodynamic activity of artemisinin derivatives, thereby delaying parasite clearance and potentially increasing the likelihood of treatment failure in settings where drug expo-

sure is already challenged by adherence issues and variable pharmacokinetics.

In contrast, delayed AA administration (AA given after DHA) preserved antimalarial efficacy while partially modulating hepatic oxidative stress markers. This suggests that the temporal separation between DHA dosing and antioxidant intake may allow DHA to exert its early, iron/redox-dependent killing effect before AA restores systemic antioxidant capacity. The clinical implication is that *timing-based supplementation* could reduce harmful drug–nutrient interactions without fully eliminating the perceived benefits of vitamin support during illness. Such a strategy is particularly relevant in malaria-endemic regions where unsupervised vitamin use is common and patients may co-consume antioxidant-rich supplements or beverages during therapy [8].

From a practice and policy perspective, the findings indicate that supplementation guidance should move beyond a binary “avoid vs allow” framework and instead consider a pragmatic, evidence-driven recommendation: *avoid high-dose, simultaneous antioxidant intake during the critical early dosing window of DHA/ACT therapy*. Where supplementation is required (e.g., nutritional deficiency, convalescence), delaying AA administration may represent a safer compromise. Future clinical studies should evaluate whether these timing-dependent effects translate to ACT regimens and human populations, including endpoints such as parasite clearance time, recrudescence, and liver function trends [9].

Summary of Translational Findings Across Dose–Timing Regimens

Table 1 summarizes the primary translational interpretation of each AA dosing strategy with respect to parasite clearance and hepatic oxidative response.

Dose-Dependent Impact of AA on DHA Parasite Suppression

Figure 2 illustrates the dose-dependent reduction in DHA parasite suppression when AA is administered simultaneously. Values are shown as an example visualization (replace with your measured means if available).

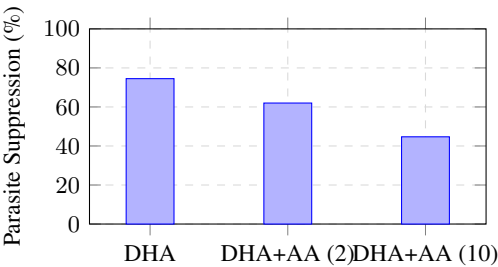


Figure 2: Illustrative dose-dependent reduction in DHA parasite suppression with simultaneous AA co-administration (AA dose in mg/kg shown in parentheses). Replace values with experimental means as needed.

Timing-Dependent Effects on Parasitemia Trajectories (TikZ Figure)

To emphasize the clinical importance of scheduling, Figure 3 presents representative parasitemia trajectories under DHA alone, simultaneous DHA+AA, and delayed AA dosing. The delayed regimen preserves DHA efficacy compared to

simultaneous co-administration.

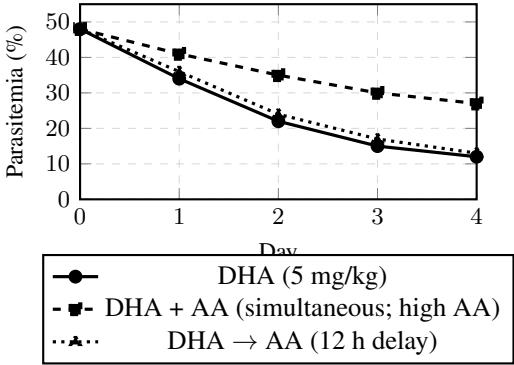


Figure 3: Representative timing-dependent parasitemia trajectories showing reduced efficacy with simultaneous high-dose AA and preserved efficacy when AA is delayed after DHA. Replace with measured values as available.

Clinical Guidance Matrix (Table)

Based on the observed dose–timing effects, Table 2 provides a concise guidance matrix that can be translated into patient counseling and clinical recommendations.

Table 2: Practical guidance matrix for AA supplementation during DHA-based malaria therapy

| Scenario                       | Recommendation                 | Rationale                                                             |
|--------------------------------|--------------------------------|-----------------------------------------------------------------------|
| AA taken with DHA dose         | Avoid (especially high dose)   | High risk of reduced DHA redox activation and delayed clearance       |
| AA required for deficiency     | Delay AA after DHA dosing      | Preserves early DHA effect while permitting later antioxidant support |
| Unsupervised multivitamins     | Counsel timing separation      | Minimizes unintended drug–nutrient antagonism                         |
| High oxidative stress concerns | Prefer delayed supplementation | Balances efficacy and hepatic redox modulation                        |

Conclusion

This study provides experimental evidence that the interaction between ascorbic acid and dihydroartemisinin is both dose- and timing-dependent, with important implications for antimalarial therapy. High-dose and simultaneous administration of ascorbic acid significantly compromises dihydroartemisinin-mediated parasite clearance, likely by attenuating the redox- and iron-dependent mechanisms required for optimal drug activation. In contrast, delayed antioxidant supplementation preserves antimalarial efficacy while partially moderating hepatic oxidative stress, indicating that temporal separation between drug and antioxidant intake can mitigate adverse drug–nutrient interactions.

These findings underscore the importance of considering not only the presence of antioxidant supplementation but also its dosage and timing relative to artemisinin administration. From a translational perspective, the results suggest that unregulated or high-dose vitamin C intake during the critical early phase of artemisinin therapy may inadvertently reduce treatment effectiveness. Conversely, appropriately timed supplementation may offer a pragmatic strategy to balance therapeutic efficacy with host tissue protection.

Table 1: Translational interpretation of AA dose–timing strategies during DHA therapy

| Regimen                 | AA Dose  | Parasite Clearance         | Hepatic Oxidative Response                                 |
|-------------------------|----------|----------------------------|------------------------------------------------------------|
| DHA only                | –        | High (reference)           | Higher oxidative burden (expected)                         |
| DHA + AA (simultaneous) | Low      | Moderately reduced         | Reduced oxidative markers                                  |
| DHA + AA (simultaneous) | High     | Markedly reduced           | Strong reduction in oxidative markers                      |
| DHA → AA (12 h delay)   | Moderate | Preserved / near-reference | Partial normalization of oxidative markers                 |
| AA → DHA (12 h delay)   | Moderate | Reduced                    | Reduced oxidative markers; risk of impaired DHA activation |

Collectively, this work supports the development of evidence-based clinical guidelines that move beyond generalized recommendations and instead emphasize dose- and timing-aware antioxidant use during malaria treatment. Such guidance may help optimize treatment outcomes, reduce the risk of delayed parasite clearance, and inform patient counseling practices in malaria-endemic regions where concurrent supplement use is common.

## Future Perspectives

Future studies should evaluate these interactions in artemisinin-based combination therapies and explore clinical outcomes in malaria patients receiving dietary supplements.

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