

Comparative Effects of Artesunate, Clindamycin and Their Combination on Parasitaemia, Body Weight and Open-Field Behaviour in *Plasmodium berghei* ANKA-Infected Wistar Rats

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Abstract

Malaria treatment studies often infer efficacy from parasite clearance, although functional responses may follow different trajectories. This study compared artesunate, clindamycin and their combination in *Plasmodium berghei* ANKA-infected Wistar rats. Sixty-four rats were allocated equally to eight infected or uninfected groups receiving vehicle, artesunate (4 mg/kg/day intraperitoneally), clindamycin (10 mg/kg/day orally), or both drugs on Days 3–7 post-inoculation. Day 3 assessments preceded the first dose. Parasitaemia was monitored on Days 3–8 in infected rats; body weight and open-field line crossing, rearing and grooming were also evaluated. Parasitaemia showed a significant time-by-treatment interaction (Greenhouse–Geisser $F(9.643, 89.998) = 366.461$, $p < 0.001$, partial $\eta^2 = 0.975$). Artesunate produced microscopically undetectable parasitaemia by Day 6, whereas clindamycin and the combination did so by Day 7. Mean Day 3–8 parasitaemia area under the curve was 45.65 ± 1.16 %·days with infected vehicle, 8.01 ± 0.22 with artesunate, 9.27 ± 0.46 with clindamycin and 7.44 ± 0.31 with combination therapy (Welch $F(3, 14.442) = 2547.698$, $p < 0.001$); every pairwise comparison was significant. Time-by-group interactions were significant for line crossing, rearing and grooming, but the combination showed no consistent behavioural advantage. Day 7 body weight differed among groups after adjustment for Day 3 weight ($F(7, 55) = 4.703$, $p < 0.001$). All animals reached the scheduled Day 8 endpoint. Artesunate provided the earliest microscopic clearance, while the combination modestly reduced cumulative parasite exposure without demonstrating pharmacological synergy or uniform behavioural benefit.

KEYWORDS: Artesunate, Clindamycin, *Plasmodium berghei* ANKA, Parasitaemia, Neurobehaviour

1. Introduction

Malaria remains a major global health challenge, with an estimated 282 million cases and 610,000 deaths worldwide in 2024 [1]. The African Region bears approximately 95% of this

burden, with Nigeria remaining among the most severely affected countries [1]. Cerebral involvement is one of the most serious complications of malaria and may be followed by persistent motor, neurobehavioural, seizure-related, and early-

and late-onset neurocognitive abnormalities among survivors [2–5]. Although human cerebral malaria is principally caused by *Plasmodium falciparum*, rodent malaria models provide controlled experimental systems for investigating parasite progression and treatment responses. However, findings from these models require cautious interpretation because no single animal model reproduces all features of human cerebral malaria [6–10].

Parenteral artesunate is the preferred treatment for severe malaria because of its rapid parasitocidal activity and established survival advantage [11]. Its principal therapeutic effect is the rapid reduction of circulating parasitaemia [12,13]. Nevertheless, parasite clearance may not immediately reverse the inflammatory, endothelial, metabolic, and neurological disturbances established during infection [9,10,14–16]. Experimental studies indicate that although artesunate can reduce parasite burden and disease severity, behavioural or neurological abnormalities may persist following parasitological rescue [7,17–20]. Thus, parasitaemia and behavioural performance should be regarded as complementary but biologically distinct indicators of treatment response.

Clindamycin possesses antiparasitic activity through inhibition of protein synthesis within the parasite apicoplast [21,22]. Its characteristic delayed-death effect results in relatively slow parasite clearance, limiting its suitability as a rapidly acting monotherapy for severe malaria [21–23]. Combining clindamycin with artesunate is pharmacologically plausible because artesunate rapidly reduces parasite load, whereas clindamycin may act against surviving parasites through a distinct cellular target. Artesunate–clindamycin regimens have previously demonstrated antimalarial efficacy in human *P. falciparum* infection, although the clinical dosing schedules and patient populations differ from the present experimental setting [24]. Evidence remains limited regarding whether adding clindamycin to artesunate reduces cumulative parasite exposure or improves short-term behavioural outcomes in *P. berghei* ANKA-infected animals. Accordingly, behavioural assessment provides an important functional endpoint for determining whether any reduction in parasite burden following adjunctive therapy is accompanied by measurable changes in neurological and behavioural function.

The open-field test provides measures of spontaneous locomotor and exploratory behaviour under standardised conditions [25]. Line crossing primarily reflects horizontal locomotor activity, whereas rearing reflects vertical exploratory activity. Grooming is a context-dependent self-directed behaviour influenced by arousal, stress, and motor capacity [25]. However, these measures are not specific to cerebral malaria and cannot, in isolation, establish cognition, neurological recovery, or a direct neuroprotective effect. Their inclusion alongside serial parasitaemia measurements nevertheless permits an exploratory

assessment of whether parasitological responses are accompanied by changes in observable behaviour.

Accordingly, this study compared artesunate, clindamycin, and their combination in infected and corresponding uninfected Wistar rat groups. The primary objective was to compare parasitaemia progression, parasite-clearance timing, and cumulative parasite exposure, measured as the area under the parasitaemia–time curve. The secondary objective was to compare changes in line crossing, rearing, and grooming between the pretreatment assessment on Day 3 and the final behavioural assessment on Day 7. By integrating serial parasitaemia with longitudinal behavioural assessment, this study sought to determine whether adjunctive clindamycin provides a measurable benefit beyond artesunate alone in reducing parasite exposure and improving short-term functional outcomes in *P. berghei* ANKA-infected rats.

2. Materials and Methods

2.1. Study Design and Setting

This controlled, parallel-group animal experiment was conducted at the Animal House Facility and Pharmacology Laboratory, Department of Pharmacology and Therapeutics, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. A total of 64 rats were randomly assigned into eight experimental groups according to infection status (infected or uninfected) and treatment intervention (vehicle, artesunate, clindamycin, or artesunate–clindamycin). The study was conducted and reported in accordance with the ARRIVE 2.0 guidelines [26].

2.2 Experimental Animals and Ethical Considerations

Sixty-four apparently healthy Wistar rats of both sexes were obtained from the Experimental Animal Breeding Unit, Department of Pharmacology and Therapeutics, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The animals had Day 0 body weights ranging from 59 to 140 g. They were acclimatised for 10 days before commencement of the experiment and housed under controlled laboratory conditions consisting of a 12-hour light–dark cycle, ambient temperature of 22–25°C, and relative humidity of 50–60%. Standard rat chow and clean drinking water were provided *ad libitum* throughout the experimental period. Both sexes were included without sex-stratified allocation. Following acclimatisation, the animals were manually randomised into eight experimental groups, with eight animals assigned to each group.

Ethical approval for the study was obtained from the Research and Ethics Committee, College of Health Sciences, Ladoke Akintola University of Technology, under approval number CHS/REC/1103. Animal handling and all experimental procedures were conducted in accordance with the institutionally approved protocol and the *Guide for the Care and Use of Laboratory Animals* [27].

2.3 Experimental Groups

The 64 animals were randomly allocated into eight groups, with eight rats per group. The uninfected groups comprised animals receiving vehicle, artesunate, clindamycin, or the combination of artesunate and clindamycin. The infected groups similarly comprised animals receiving vehicle, artesunate, clindamycin, or the artesunate–clindamycin combination. Thus, the experimental groups consisted of uninfected + vehicle, uninfected + artesunate, uninfected + clindamycin, uninfected + artesunate and clindamycin, infected + vehicle, infected + artesunate, infected + clindamycin, and infected + artesunate and clindamycin.

Plasmodium berghei, ANKA strain was obtained from the Institute for Advanced Medical Research and Training (IAMRAT), University of Ibadan and maintained by passage in mice. Infection in the donor rat was confirmed microscopically using Giemsa-stained thin blood films. The donor parasitaemia used for inoculum preparation was 16.7%. The concentration of parasitised erythrocytes was calculated from the measured parasitaemia and erythrocyte count. The infected blood was subsequently diluted to prepare a standardised inoculum containing 1×10^6 parasitised erythrocytes in 0.2 mL. The inoculum was prepared immediately before administration, and parasite stages were neither separately synchronised nor quantified.

On Day 0, each rat assigned to an infected group received 0.2 mL of the standardised inoculum by intraperitoneal injection. Animals assigned to the four uninfected groups did not receive parasitised erythrocytes. Establishment of infection was confirmed 72 hours after inoculation by microscopic examination of Giemsa-stained blood films. Parasitaemia was confirmed before commencement of treatment, and all pre-treatment assessments were completed before administration of the first treatment dose.

2.4 Drug Preparation and Treatment

Treatment commenced on Day 3 post-inoculation. Artesunate was prepared at a working concentration of 1 mg/mL and administered intraperitoneally at a dose of 4 mg/kg once daily, whereas clindamycin was prepared at a working concentration of 2 mg/mL and administered orally at 10 mg/kg once daily. Treatment was continued for five consecutive days, from Day 3 through Day 7 post-inoculation. Animals assigned to combination therapy received artesunate and clindamycin separately through the intraperitoneal and oral routes, respectively. The volume of drug administered to each animal was calculated according to its body weight. Body weight was recorded on Days 0, 3, 5, and 7, and treatment volumes were adjusted using the most recent body-weight measurement. The uninfected and infected vehicle-control groups received the corresponding vehicles without active drug. All Day 3 pre-treatment measurements, including parasitaemia assessment and

open-field behavioural testing, were completed before administration of the first treatment dose. The final treatment dose was administered on Day 7, and terminal sample collection was performed on Day 8.

2.5 Parasitaemia Assessment and Definition of Parasite Clearance

Parasitaemia was assessed daily from Day 3 to Day 8 post-inoculation. The Day 3 assessment was performed before administration of the first treatment dose and was therefore designated as the pretreatment parasitaemia measurement. A small quantity of blood was collected from the tail vein after the sampling site had been cleaned with 70% ethanol. A thin blood film was prepared on a clean, grease-free glass slide, allowed to air-dry, and fixed in absolute methanol for approximately two minutes. The fixed film was stained with freshly prepared Giemsa working solution, rinsed with buffered water, and allowed to air-dry.

Each stained blood film was examined by light microscopy using the $\times 100$ oil-immersion objective. Parasitised and non-parasitised erythrocytes were counted systematically across multiple microscopic fields, with approximately 1,000 erythrocytes evaluated per film. Technically inadequate or poorly stained films were repeated where necessary. Microscopic assessment and parasite counting were performed by experimenters who were blinded to the experimental groupings. The microscopy procedure followed established principles for the preparation, staining, and examination of malaria blood films [28].

Percentage parasitaemia was calculated as the number of parasitised erythrocytes divided by the total number of erythrocytes counted, multiplied by 100. Daily animal-level parasitaemia values were retained for longitudinal analysis and calculation of the area under the parasitaemia–time curve. For the purposes of this study, parasite clearance was operationally defined as the first scheduled assessment day on which no parasitised erythrocyte was detected in the examined blood film. A recorded parasitaemia value of zero therefore represented microscopic non-detection under the study procedure and was not interpreted as evidence of sterile parasitological cure.

2.6 Body Weight and Clinical Monitoring

Body weight was measured in all 64 animals on Days 0, 3, 5, and 7 using a calibrated weighing balance. Day 0 represented the pre-inoculation measurement, while the Day 3 measurement was obtained before administration of the first treatment dose. Measurements on Days 5 and 7 were similarly obtained before the scheduled drug administration. The recorded body weights were used to calculate weight-adjusted treatment volumes. Treatment-period percentage change in body weight was calculated from Day 3 to Day 7 using the formula: percentage weight change = $[(\text{Day 7 weight} - \text{Day 3 weight}) / \text{Day 3 weight}] \times 100$. Animals were observed daily from inoculation until the

planned Day 8 endpoint for general clinical condition and mortality. Survival was monitored as a safety observation and was not designated as a separate efficacy outcome.

2.7 Open-Field Neurobehavioural Assessment

Neurobehavioural performance was assessed using the open-field test on Days 3 and 7 post-inoculation. The Day 3 assessment was conducted immediately before administration of the first treatment dose and therefore served as the post-infection pretreatment reference. The same animals were reassessed on Day 7. Behavioural testing was conducted during the light phase under standardised laboratory conditions.

The open-field apparatus consisted of a square enclosure with opaque walls and a floor divided into equal, permanently marked squares. Each rat was assessed individually for a 10-minute observation period, during which the frequencies of line crossing, rearing, and grooming were recorded. Behavioural scoring was performed by experimenters who were blinded to the experimental groupings. A line crossing was recorded when all four paws crossed a marked boundary. Rearing was defined as standing on the hind limbs, with or without support from the enclosure wall. Grooming included face washing, body grooming, licking, and scratching. These behavioural outcomes were considered indicators of locomotor, exploratory, and self-directed activity, respectively, rather than direct measures of cerebral pathology [29]. For each behavioural outcome, a change score was calculated by subtracting the Day 3 value from the corresponding Day 7 value. A positive change score indicated increased activity, whereas a negative score indicated decreased activity over the treatment period.

2.8 Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 27.0 (IBM Corporation, Armonk, NY, USA). Continuous variables were expressed as mean $\pm$ standard deviation. Distributional assumptions were assessed using the Shapiro–Wilk test, Q–Q plots, and boxplots, while homogeneity of variance was evaluated using Levene’s test. All statistical tests were two-sided, and statistical significance was set at $p < 0.05$. Probability values displayed by SPSS as .000 were reported as $p < 0.001$.

Parasitaemia analyses were restricted to the 32 infected rats. Changes in parasitaemia from Days 3 to 8 were evaluated using repeated-measures analysis of variance, with time specified as the within-subject factor and treatment group as the between-subject factor. Greenhouse–Geisser-adjusted results were used when the assumption of sphericity was violated, and effect sizes were expressed as partial eta squared. Because the daily parasitaemia data demonstrated unequal variances, group differences on Days 3–5 were evaluated using Welch’s analysis of variance followed by Games–Howell pairwise comparisons. Parasitaemia on Days 6–8 was analysed using the Kruskal–Wallis test with Bonferroni-adjusted pairwise comparisons

because treatment-related parasite clearance resulted in zero values and zero within-group variance. Individual parasitaemia area under the curve from Days 3 to 8 was calculated using the trapezoidal method and compared among the infected groups using Welch’s analysis of variance followed by Games–Howell post hoc tests.

Day 7 body weight was compared across the eight experimental groups using analysis of covariance, with Day 3 body weight included as the covariate. Homogeneity of regression slopes was examined using the body-weight-by-group interaction before fitting the final model. Adjusted group means were compared using Bonferroni correction. Because the model residuals were not normally distributed, percentage change in body weight from Day 3 to Day 7 was additionally evaluated using the Kruskal–Wallis test with Bonferroni-adjusted pairwise comparisons as a sensitivity analysis.

Line crossing, rearing, and grooming were analysed separately using repeated-measures general linear models, with assessment time (Day 3 and Day 7) specified as the within-subject factor and experimental group as the between-subject factor. The main effects of time and group, as well as the time-by-group interaction, were evaluated, with partial eta squared reported as the measure of effect size. For each behavioural outcome, a change score was also calculated as the Day 7 value minus the Day 3 value. Differences in change scores among groups were assessed using one-way analysis of variance, with Welch’s robust test and Games–Howell pairwise comparisons applied where variance heterogeneity was present.

3. Result

3.1 Animal Retention and Body-Weight Changes

All 64 rats completed the experiment through Day 8, with eight animals retained in each experimental group and no mortality recorded before sacrifice. Body weight on Day 0 ranged from 59 to 140 g, with an overall mean of 89.33 ± 18.31 g. The overall mean body weight increased to 92.34 ± 19.61 g on Day 3 and 101.98 ± 21.18 g on Day 7. After adjustment for Day 3 body weight, a significant difference in Day 7 body weight was observed among the experimental groups (ANCOVA: $F(7,55) = 4.703$, $p < 0.001$, partial $\eta^2 = 0.374$). Day 3 body weight was a strong predictor of Day 7 body weight ($F(1,55) = 541.037$, $p < 0.001$, partial $\eta^2 = 0.908$). The adjusted mean Day 7 body weights were 104.53 g for the uninfected vehicle group, 106.03 g for the uninfected artesunate group, 102.93 g for the uninfected clindamycin group, 103.80 g for the uninfected combination group, 105.59 g for the infected vehicle group, 95.55 g for the infected artesunate group, 101.22 g for the infected clindamycin group, and 96.22 g for the infected combination group. Bonferroni-adjusted pairwise comparisons showed that the infected artesunate group had a significantly lower adjusted Day 7 body weight than the uninfected vehicle group ($p = 0.027$), uninfected artesunate group ($p = 0.005$), and

infected vehicle group ($p = 0.007$). The infected combination group also had a significantly lower adjusted Day 7 body weight than the infected vehicle group ($p = 0.019$). No other adjusted pairwise comparisons reached statistical significance. The body-weight findings are summarised in **Table 1**. Sensitivity analysis of percentage change in body weight from Day 3 to Day 7 also demonstrated a significant overall group difference (Kruskal–Wallis $H(7) = 26.468$, $p < 0.001$). Following Bonferroni adjustment, percentage weight gain was significantly lower in the infected artesunate group than in the uninfected artesunate group (adjusted $p = 0.003$) and significantly lower in the infected combination group than in the uninfected artesunate group (adjusted $p = 0.010$).

3.2 Parasitaemia Progression and Parasite Clearance

Daily parasitaemia and cumulative parasite burden are presented in **Table 2** and **Figure 1**. Parasitaemia was confirmed in all infected groups on Day 3 before administration of the first treatment dose. Mean Day 3 parasitaemia was $5.55 \pm 0.22\%$ in the infected vehicle group, $5.90 \pm 0.62\%$ in the infected artesunate group, $5.80 \pm 0.16\%$ in the infected clindamycin group, and $5.54 \pm 0.23\%$ in the infected combination group. Although the overall Welch test indicated a significant difference among groups ($p = 0.040$), none of the Games–Howell pairwise comparisons reached statistical significance, indicating no clearly identifiable pairwise pretreatment differences among the infected groups. Mauchly's test indicated violation of the sphericity assumption ($W = 0.119$, $\chi^2(14) = 55.519$, $p < 0.001$); consequently, Greenhouse–Geisser-adjusted results were interpreted. Parasitaemia changed significantly over time ($F(3.214, 89.998) = 305.161$, $p < 0.001$, partial $\eta^2 = 0.916$), and a significant time-by-treatment-group interaction was observed ($F(9.643, 89.998) = 366.461$, $p < 0.001$, partial $\eta^2 = 0.975$). These findings indicate that the trajectory of parasitaemia from Days 3 to 8 differed substantially according to treatment group.

In the infected vehicle group, parasitaemia increased progressively from $5.55 \pm 0.22\%$ on Day 3 to $6.79 \pm 0.61\%$ on Day 4, $8.83 \pm 0.84\%$ on Day 5, $9.58 \pm 0.37\%$ on Day 6, $11.00 \pm 0.33\%$ on Day 7, and $13.34 \pm 0.93\%$ on Day 8. In contrast, parasitaemia declined following treatment in all three treated groups. On Day 4, the artesunate–clindamycin combination group had significantly lower parasitaemia than both the artesunate group ($p = 0.001$) and the clindamycin group ($p = 0.036$), whereas no significant difference was observed between the artesunate and clindamycin groups ($p = 0.997$). All three treatment groups had significantly lower Day 4 parasitaemia than the infected vehicle group (all $p < 0.001$). By Day 5, mean parasitaemia had declined to $1.33 \pm 0.23\%$ in the artesunate group, $1.85 \pm 0.23\%$ in the clindamycin group, and $1.15 \pm 0.06\%$ in the combination group, compared with $8.83 \pm 0.84\%$ in the infected vehicle group. Artesunate

produced significantly lower Day 5 parasitaemia than clindamycin ($p = 0.002$), whereas the difference between artesunate and combination therapy was not statistically significant ($p = 0.212$). The combination group had significantly lower parasitaemia than the clindamycin group ($p < 0.001$).

By Day 6, no parasites were detected in the artesunate group, whereas residual parasitaemia remained detectable in the clindamycin group ($0.81 \pm 0.05\%$) and the combination group ($0.38 \pm 0.28\%$). The Day 6 distributions differed significantly among the infected groups (Kruskal–Wallis $H(3) = 28.258$, $p < 0.001$). Parasitaemia remained undetectable in all three treatment groups on Days 7 and 8, whereas parasitaemia continued to increase in the infected vehicle group. The distributions on both Day 7 and Day 8 differed significantly among groups ($H(3) = 30.175$, $p < 0.001$ for each day). Following Bonferroni adjustment, the infected vehicle group differed significantly from each of the treated groups on both days (all adjusted $p < 0.001$), whereas no significant differences were observed among the three treated groups.

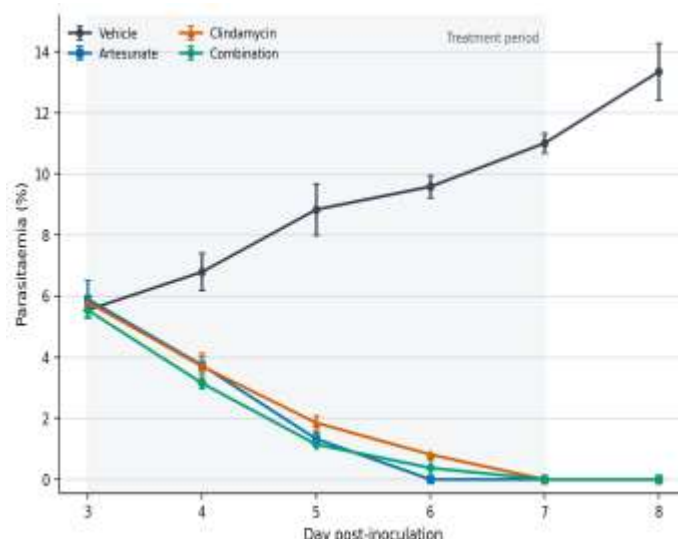


Figure 1. Parasitaemia progression from Days 3–8 in infected rats. Values are mean $\pm$ SD ($n = 8$ per group). The shaded region denotes the treatment period (Days 3–7). Day 3 measurements preceded the first dose; 0% indicates no parasites detected by the study microscopy procedure.

3.3 Cumulative Parasitaemia Burden

Cumulative parasitaemia burden from Days 3 to 8 differed significantly among the infected groups. Because the assumption of homogeneity of variance was violated (Levene's test, $p = 0.027$), Welch's robust analysis was used. A significant treatment-group effect was observed for the parasitaemia area under the curve (Welch $F(3, 14.442) = 2547.698$, $p < 0.001$). The infected vehicle group had the highest parasitaemia area under the curve, with a mean value of 45.65 ± 1.16 percentage-parasitaemia-days. Substantially lower values were observed in the artesunate (8.01 ± 0.22)

Table 1. Body weight by experimental group and adjusted Day 7 comparisons

Group	Day 0 (g)	Day 3 (g)	Day 5 (g)	Day 7 (g)	Adjusted Day 7 mean (95% CI)	Day 3–7 change (%)
G1: Uninfected + Vehicle	69.88 ± 9.51	76.25 ± 13.44	80.00 ± 12.60	86.75 ± 19.65	104.53 (100.69–108.37)	12.92 ± 6.90
G2: Uninfected + Artesunate	76.38 ± 9.68	75.12 ± 7.66	84.12 ± 8.22	87.00 ± 8.54	106.03 (102.14–109.91)	15.96 ± 6.47
G3: Uninfected + Clindamycin	82.25 ± 4.98	88.00 ± 6.21	92.38 ± 5.76	98.12 ± 8.69	102.92 (99.38–106.47)	11.44 ± 4.58
G4: Uninfected + Artesunate + Clindamycin	106.00 ± 8.75	99.88 ± 8.18	104.50 ± 9.93	112.12 ± 8.66	103.80 (100.21–107.40)	12.36 ± 3.55
G5: Infected + Vehicle	96.75 ± 18.75	104.25 ± 18.34	116.12 ± 21.31	118.75 ± 22.48	105.59 (101.90–109.29)	13.72 ± 6.53
G6: Infected + Artesunate	86.38 ± 15.26	90.38 ± 15.46	93.62 ± 17.18	93.38 ± 18.49	95.55 (92.03–99.08)	3.14 ± 7.29
G7: Infected + Clindamycin	89.50 ± 12.00	84.00 ± 9.20	92.12 ± 8.59	92.00 ± 9.59	101.22 (97.61–104.83)	9.65 ± 4.15
G8: Infected + Artesunate + Clindamycin	107.50 ± 23.56	120.88 ± 23.61	130.88 ± 20.11	127.75 ± 23.52	96.22 (91.78–100.67)	5.91 ± 3.21

Raw values and percentage changes are mean ± SD ($n = 8$). Adjusted Day 7 means were estimated by ANCOVA at Day 3 weight = 92.344 g. Group effect: $F(7,55) = 4.703$, $p < 0.001$, partial $\eta^2 = 0.374$. Bonferroni comparisons: $G6 < G1$ ($p = 0.027$), $G2$ ($p = 0.005$) and $G5$ ($p = 0.007$); $G8 < G5$ ($p = 0.019$). Percentage-change sensitivity analysis: $H(7) = 26.468$, $p < 0.001$; $G6 < G2$ (adjusted $p = 0.003$) and $G8 < G2$ (adjusted $p = 0.010$).

Table 2. Parasitaemia progression and Day 3–8 area under the curve in infected rats

Treatment group	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	AUC (%-days)
Infected + Vehicle	5.55 ± 0.22 ^a	6.79 ± 0.61 ^a	8.83 ± 0.84 ^a	9.58 ± 0.37 ^a	11.00 ± 0.33 ^a	13.34 ± 0.93 ^a	45.65 ± 1.16 ^a
Infected + Artesunate	5.90 ± 0.62 ^a	3.74 ± 0.27 ^b	1.33 ± 0.23 ^c	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	8.01 ± 0.22 ^c
Infected + Clindamycin	5.80 ± 0.16 ^a	3.70 ± 0.44 ^b	1.85 ± 0.23 ^b	0.81 ± 0.05 ^{ac}	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	9.27 ± 0.46 ^b
Infected + Artesunate + Clindamycin	5.54 ± 0.23 ^a	3.15 ± 0.19 ^c	1.15 ± 0.06 ^c	0.38 ± 0.28 ^{bc}	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	7.44 ± 0.31 ^d

Values are mean ± SD ($n = 8$). Within a column, values sharing at least one superscript letter did not differ after multiplicity adjustment. Day 3–5: Games–Howell; Day 6–8: Kruskal–Wallis pairwise comparisons with Bonferroni adjustment; AUC: Games–Howell. A value of 0.00 denotes no parasites detected by the study microscopy procedure.

clindamycin (9.27 ± 0.46), and artesunate–clindamycin combination (7.44 ± 0.31) groups.

Games–Howell pairwise comparisons showed that each treatment significantly reduced cumulative parasitaemia relative to the infected vehicle group (all $p < 0.001$). Combination therapy produced a significantly lower cumulative parasite burden than artesunate monotherapy (mean difference = -0.57 , $p = 0.005$) and clindamycin monotherapy (mean difference = -1.83 , $p < 0.001$). Artesunate monotherapy also produced a significantly lower cumulative parasite burden than clindamycin monotherapy (mean difference = -1.26 , $p < 0.001$). Thus, although artesunate monotherapy achieved the earliest complete microscopic parasite clearance, the artesunate–clindamycin combination produced the lowest overall cumulative parasitaemia burden.

3.4 Open-Field Performance: Line-Crossing Activity

Line-crossing frequency decreased significantly overall between Day 3 and Day 7 (time effect: $F(1,56) = 25.467$, $p < 0.001$, partial $\eta^2 = 0.313$). However, the magnitude and direction of change varied significantly among the experimental groups, as demonstrated by a significant time-by-group interaction ($F(7,56) = 4.748$, $p < 0.001$, partial $\eta^2 = 0.372$). A significant overall group effect was also observed ($F(7,56) = 28.834$, $p < 0.001$, partial $\eta^2 = 0.783$).

Mean line-crossing frequency in the uninfected vehicle group decreased from 56.38 ± 18.45 on Day 3 to 49.75 ± 7.01 on Day 7, while the uninfected artesunate group decreased from 55.75 ± 8.88 to 46.88 ± 13.44 . In contrast, line crossing increased from 76.63 ± 11.31 to 94.13 ± 10.54 in the uninfected clindamycin group and decreased from 59.38 ± 5.66 to 49.63 ± 7.21 in the uninfected combination group. Among the infected groups, line crossing decreased from 41.75 ± 10.22 to 20.25 ± 7.70 in the vehicle group, from 64.38 ± 20.49 to 42.00 ± 14.48 in the artesunate group, from 49.63 ± 17.41 to 22.88 ± 14.11 in the clindamycin group, and from 44.00 ± 13.66 to 32.25 ± 8.92 in the combination group.

The mean Day 7-minus-Day 3 change scores were -6.63 ± 24.14 , -8.88 ± 11.52 , 17.50 ± 14.81 , -9.75 ± 7.23 , -21.50 ± 11.63 , -22.38 ± 20.66 , -26.75 ± 26.42 , and -11.75 ± 17.44 for Groups 1–8, respectively. Group differences in change scores remained significant using Welch’s robust test ($F(7,23.590) = 5.107$, $p = 0.001$). Games–Howell comparisons showed that the increase observed in the uninfected clindamycin group differed significantly from the changes observed in the uninfected artesunate ($p = 0.024$), uninfected combination ($p = 0.013$), infected vehicle ($p = 0.001$), infected artesunate ($p = 0.012$), infected clindamycin ($p = 0.025$), and infected combination ($p = 0.044$) groups. However, no statistically significant differences in line-crossing change were observed among the four infected groups.

Table 3A. Open-field line-crossing counts

Group		Day 3	Day 7	Change (Day 7 – Day 3)
G1: Uninfected Vehicle	+	56.38 ± 18.45	49.75 ± 7.01	-6.63 ± 24.14
G2: Uninfected Artesunate	+	55.75 ± 8.88	46.88 ± 13.44	-8.88 ± 11.52
G3: Uninfected Clindamycin	+	76.63 ± 11.31	94.13 ± 10.54	17.50 ± 14.81
G4: Uninfected Artesunate + Clindamycin	+	59.38 ± 5.66	49.63 ± 7.21	-9.75 ± 7.23
G5: Infected Vehicle	+	41.75 ± 10.22	20.25 ± 7.70	-21.50 ± 11.63
G6: Infected Artesunate	+	64.38 ± 20.49	42.00 ± 14.48	-22.38 ± 20.66
G7: Infected Clindamycin	+	49.63 ± 17.41	22.88 ± 14.11	-26.75 ± 26.42
G8: Infected Artesunate + Clindamycin	+	44.00 ± 13.66	32.25 ± 8.92	-11.75 ± 17.44

Values are mean $\pm$ SD ($n = 8$). Time $\times$ group: $F(7,56) = 4.748$, $p < 0.001$, partial $\eta^2 = 0.372$. No Games–Howell comparison of change scores among the four infected groups was significant.

3.5 Open-Field Performance: Rearing Activity

Rearing frequency decreased significantly overall between Day 3 and Day 7 (time effect: $F(1,56) = 14.123$, $p < 0.001$, partial $\eta^2 = 0.201$). A significant time-by-group interaction was also observed, indicating that changes in rearing frequency differed among the experimental groups ($F(7,56) = 14.961$, $p < 0.001$, partial $\eta^2 = 0.652$). The overall group effect was significant ($F(7,56) = 20.471$, $p < 0.001$, partial $\eta^2 = 0.719$).

In the uninfected vehicle group, mean rearing frequency increased from 11.25 ± 3.88 on Day 3 to 19.38 ± 4.57 on Day 7, while the uninfected clindamycin group increased from 20.25 ± 6.14 to 29.88 ± 5.59 . Rearing decreased from 26.63 ± 5.71 to 12.25 ± 4.56 in the uninfected artesunate group, whereas little change was observed in the uninfected combination group, from 25.75 ± 3.24 to 26.75 ± 11.60 . Among the infected animals, mean rearing frequency changed from 11.75 ± 1.91 to 10.38 ± 4.98 in the vehicle group, from 22.50 ± 4.47 to 9.13 ± 3.31 in the artesunate group, from 15.63 ± 4.00 to 7.00 ± 5.10 in the clindamycin group, and from 11.63 ± 5.73 to 5.63 ± 2.26 in the combination group.

The mean Day 7-minus-Day 3 change scores for Groups 1–8 were 8.13 ± 6.18 , -14.37 ± 4.87 , 9.63 ± 6.39 , 1.00 ± 12.08 , -1.37 ± 5.13 , -13.37 ± 5.93 , -8.62 ± 3.50 , and -6.00 ± 5.63 , respectively. Change scores differed significantly among groups using Welch’s robust test ($F(7,23.837) = 16.919$, $p < 0.001$). Among the infected groups, the decline in rearing was significantly greater in the artesunate group than in the infected vehicle group (Games–Howell mean difference = -12.00 , $p = 0.012$). No other pairwise differences in rearing change among the infected groups reached statistical significance following multiple-comparison adjustment.

Table 3B. Open-field rearing counts

Group	Day 3	Day 7	Change (Day 7 – Day 3)
G1: Uninfected + Vehicle	11.25 ± 3.88	19.38 ± 4.57	8.13 ± 6.18
G2: Uninfected + Artesunate	26.63 ± 5.71	12.25 ± 4.56	-14.37 ± 4.87
G3: Uninfected + Clindamycin	20.25 ± 6.14	29.88 ± 5.59	9.63 ± 6.39
G4: Uninfected + Artesunate + Clindamycin	25.75 ± 3.24	26.75 ± 11.60	1.00 ± 12.08
G5: Infected + Vehicle	11.75 ± 1.91	10.38 ± 4.98	-1.37 ± 5.13
G6: Infected + Artesunate	22.50 ± 4.47	9.13 ± 3.31	-13.37 ± 5.93
G7: Infected + Clindamycin	15.63 ± 4.00	7.00 ± 5.10	-8.62 ± 3.50
G8: Infected + Artesunate + Clindamycin	11.63 ± 5.73	5.63 ± 2.26	-6.00 ± 5.63

Values are mean ± SD (n = 8). Time × group: $F(7,56) = 14.961$, $p < 0.001$, partial $\eta^2 = 0.652$. Among infected groups, G6 declined more than G5 (Games–Howell $p = 0.012$); other infected-group comparisons were not significant.

3.6 Open-Field Performance: Grooming Behaviour

Grooming frequency did not change significantly overall between Day 3 and Day 7 (time effect: $F(1,56) = 0.650$, $p = 0.423$, partial $\eta^2 = 0.011$). However, the significant time-by-group interaction indicated that the pattern of change in grooming differed among the experimental groups ($F(7,56) = 7.587$, $p < 0.001$, partial $\eta^2 = 0.487$). A significant overall group effect was also observed ($F(7,56) = 10.770$, $p < 0.001$, partial $\eta^2 = 0.574$). Mean grooming frequency increased from 6.00 ± 0.54 on Day 3 to 8.50 ± 2.56 on Day 7 in the uninfected vehicle group, while it decreased from 9.38 ± 2.45 to 6.25 ± 1.17 in the uninfected artesunate group. The uninfected clindamycin group showed an increase from 6.50 ± 0.54 to 10.13 ± 3.83 , whereas grooming decreased from 7.63 ± 2.13 to 4.00 ± 1.51 in the uninfected combination group. Among infected animals, grooming frequency changed from 4.50 ± 1.77 to 3.75 ± 2.38 in the vehicle group, from 4.25 ± 1.28 to 9.50 ± 2.33 in the artesunate group, from 5.13 ± 1.25 to 4.50 ± 2.33 in the clindamycin group, and from 4.50 ± 3.89 to 3.88 ± 0.84 in the combination group.

The mean Day 7-minus-Day 3 change scores for Groups 1–8 were 2.50 ± 2.67 , -3.12 ± 3.31 , 3.63 ± 4.27 , -3.62 ± 2.45 , -0.75 ± 3.54 , 5.25 ± 2.77 , -0.62 ± 2.72 , and -0.62 ± 3.85 , respectively. Change scores differed significantly among the groups using Welch's robust test ($F(7,23.941) = 8.208$, $p < 0.001$). Among the infected groups, the increase in grooming frequency observed in the artesunate group was significantly greater than the changes observed in the infected vehicle group ($p = 0.034$) and infected clindamycin group ($p = 0.013$). The difference between the infected artesunate and infected combination groups did not reach statistical significance following adjustment ($p = 0.057$), while no significant difference was observed between the infected clindamycin and infected combination groups ($p = 1.000$).

Table 3C. Open-field grooming counts

Group	Day 3	Day 7	Change (Day 7 – Day 3)
G1: Uninfected + Vehicle	6.00 ± 0.54	8.50 ± 2.56	2.50 ± 2.67
G2: Uninfected + Artesunate	9.38 ± 2.45	6.25 ± 1.17	-3.12 ± 3.31
G3: Uninfected + Clindamycin	6.50 ± 0.54	10.13 ± 3.83	3.63 ± 4.27
G4: Uninfected + Artesunate + Clindamycin	7.63 ± 2.13	4.00 ± 1.51	-3.62 ± 2.45
G5: Infected + Vehicle	4.50 ± 1.77	3.75 ± 2.38	-0.75 ± 3.54
G6: Infected + Artesunate	4.25 ± 1.28	9.50 ± 2.33	5.25 ± 2.77
G7: Infected + Clindamycin	5.13 ± 1.25	4.50 ± 2.33	-0.62 ± 2.72
G8: Infected + Artesunate + Clindamycin	4.50 ± 3.89	3.88 ± 0.84	-0.62 ± 3.85

Values are mean ± SD (n = 8). Time × group: $F(7,56) = 7.587$, $p < 0.001$, partial $\eta^2 = 0.487$. Among infected groups, the increase in G6 exceeded G5 ($p = 0.034$) and G7 ($p = 0.013$); G6 versus G8 was not significant ($p = 0.057$).

Discussion

This study evaluated the effects of artesunate, clindamycin, and their combination on parasitaemia and parasite clearance in infected animals. The treatment was associated with a marked reduction in parasitaemia compared with the infected vehicle group, although the magnitude and timing of the response differed among treatment groups. Artesunate produced the most rapid microscopic clearance, clindamycin showed a slower response, and the combination produced the lowest cumulative parasitaemia burden. These findings support considering the distinct pharmacodynamic profiles of the two drugs and the potential value of combining them.

Before treatment, Games–Howell comparisons did not identify significant pairwise differences among the infected groups on Day 3, indicating no clear evidence of pre-treatment parasitaemia imbalance. This finding should not, however, be interpreted as proof of statistical equivalence. After treatment commenced, parasitaemia increased progressively in the infected vehicle group but declined markedly in all treated groups, demonstrating the antiplasmodial effects of artesunate, clindamycin and their combination.

Artesunate produced the most rapid response, with parasitaemia becoming microscopically undetectable by Day 6. Artemisinin derivatives produce steep parasite-clearance profiles and are consequently used as the rapid-acting components of artemisinin-based combination therapies [1, 2, 12, 13]. Artesunate has similarly rapid therapeutic effects in experimental cerebral malaria, supporting the biological plausibility of the response observed in the present study [7]. Parasite-clearance rates nevertheless reflect both drug-mediated parasite killing and host-dependent removal of parasitised erythrocytes; microscopy therefore measures the disappearance

of detectable circulating parasites rather than proof of sterile cure [12, 13].

Clindamycin produced a slower response, with residual parasitaemia persisting on Day 6 before becoming microscopically undetectable on Day 7. Clindamycin inhibits prokaryotic-type protein translation within the parasite apicoplast [21, 22]. Its antiparasmodial action commonly exhibits a delayed-death phenotype in which initially treated parasites may complete the current erythrocytic cycle, whereas their progeny failed to develop normally during the subsequent cycle [21–23]. The observed lag in parasite clearance is therefore consistent with clindamycin's established mechanism rather than evidence of therapeutic inactivity.

The artesunate–clindamycin combination also produced a marked parasitological response and generated the lowest cumulative parasitaemia burden. Its area under the curve was significantly lower than those of both monotherapies, although the absolute difference from artesunate was modest. Moreover, artesunate monotherapy achieved microscopically undetectable parasitaemia one day earlier than the combination. The combination therefore improved cumulative parasite suppression but did not accelerate the time to microscopic clearance beyond that achieved with artesunate alone. The different pharmacodynamic profiles of the two drugs provide a plausible basis for a complementary effect: artesunate rapidly reduces the circulating parasite biomass, whereas clindamycin contributes a slower apicoplast-directed action [12, 21, 22]. This rationale aligns with the established use of a rapidly acting antimalarial alongside a slower-acting partner drug to sustain parasite suppression [1, 2]. The clinical plausibility of combining artesunate with clindamycin is supported by a randomised trial in which artesunate–clindamycin produced significantly shorter parasite- and fever-clearance times than quinine–clindamycin in children with uncomplicated falciparum malaria, while the final cure rates were comparable [24]. This evidence supports the feasibility of the drug combination but does not establish direct clinical equivalence with the present findings. Experimental *Plasmodium berghei* infection differs from human *Plasmodium falciparum* malaria in parasite biology, host response, disease progression and treatment exposure; translation must therefore remain cautious [6, 8].

The conclusion drawn from this study is that artesunate–clindamycin enhanced cumulative parasite suppression, while artesunate monotherapy retained the advantage of the fastest microscopic clearance. The findings support complementarity between the treatments but do not establish pharmacological synergy or the combination's universal superiority across all parasitological endpoints.

Body weight provides a useful but nonspecific indication of the general health and welfare of experimental animals [27]. The observed clinical changes in body weight require a cautious

interpretation because changes in body weight may reflect altered food intake, hydration, metabolism, infection severity, experimental stress, or treatment tolerability [26, 27].

In the present study, percentage weight change during the treatment period differed significantly among the groups. However, every group recorded a positive mean weight change, and no experimental rat died before Day 8. These findings indicate that overt group-level wasting or early mortality did not occur during the observation period. Nevertheless, maintenance of body weight alone does not exclude transient illness or other physiological disturbances [27].

Experimental *Plasmodium berghei* ANKA infection has been associated with progressive weight loss in susceptible rodent models [8]. The magnitude of this response may vary with the rodent species, parasite–host combination, inoculum and duration of observation [8]. In the present study, the infected vehicle group gained weight, whereas the infected artesunate and infected artesunate–clindamycin groups showed comparatively smaller gains. Infection alone therefore cannot fully explain the reduced weight gain observed in the artesunate-containing groups.

Systemic infection and inflammatory signalling may reduce feeding, alter spontaneous activity and produce sickness-related behaviour [31]. Consequently, behavioural reductions during infection may reflect a combination of systemic illness and neurological or motivational changes rather than an isolated motor deficit [31]. Because we did not quantify feed and water consumption, we could not determine their possible contributions to the observed weight patterns.

The smaller weight gains in the artesunate-containing infected groups should not be interpreted as evidence of drug toxicity. Establishing treatment-related toxicity would require corroborating clinical, biochemical or histopathological evidence in addition to body-weight measurements [27]. ARRIVE 2.0 similarly recommends reporting body-weight changes alongside clinical monitoring, adverse events, and humane-endpoint observations [26]. Body weight should therefore be treated as a secondary indicator of systemic condition and treatment tolerability rather than as independent evidence of behavioural recovery, therapeutic efficacy or toxicity.

Line-crossing frequency provides an index of spontaneous locomotor and horizontal exploratory activity in the open-field test [25, 29]. It is not specific to neurological integrity because performance may also be influenced by anxiety, motivation, fatigue, habituation and systemic illness [25, 31, 32]. Changes in line crossing should therefore be interpreted as alterations in overall activity rather than direct evidence of cerebral injury or neuroprotection.

Although line-crossing activity declined over time and the time-by-group interaction was significant, the pattern did not

demonstrate a consistent treatment-related benefit among infected rats. All infected groups showed reduced activity between Days 3 and 7, and none of the treated groups differed significantly from the infected vehicle group in the Games–Howell comparisons. The numerically smaller decline in the combination group consequently cannot be interpreted as preservation or restoration of locomotor activity.

The interaction appeared to be influenced partly by increased line crossing in the uninfected clindamycin group, whereas most other groups showed reductions. This divergent response indicates that the omnibus interaction was not driven primarily by behavioural recovery in the infected treatment groups. Repeated exposure to an open-field arena can itself reduce exploration through habituation and increasing familiarity with the test environment [25,29]. Systemic sickness and inflammatory signalling may further suppress spontaneous activity independently of permanent neurological damage [31]. The absence of behavioural improvement despite marked parasite clearance suggests that parasitological and functional recovery did not occur in parallel. Persistent motor and cognitive abnormalities have been reported after successful antimalarial treatment in murine cerebral malaria, indicating that removal of circulating parasites may not immediately reverse established inflammatory or neurological disturbances [33,36]. Conversely, locomotor impairment has not been consistently demonstrated in less severe experimental malaria, showing that behavioural expression varies with infection severity, host characteristics and assessment timing [34].

These findings therefore provide evidence of group-dependent changes in spontaneous locomotor activity but not of treatment-induced neurobehavioural recovery. They also do not establish equivalence among the infected treatments, because nonsignificant pairwise comparisons indicate an absence of detectable differences rather than proof that the treatment effects were identical.

Rearing reflects vertical exploration and responsiveness to environmental novelty, but it is sensitive to stress, habituation and general motor capacity [25,35]. It should therefore be considered alongside other behavioural measures rather than treated as an isolated marker of neurological function. The significant time-by-group interaction for rearing indicated that the experimental groups followed different behavioural trajectories. However, none of the treatments significantly improved relative to the infected vehicle group. Artesunate was associated with a significantly greater decline than the infected vehicle group, whereas clindamycin and combination therapy did not differ significantly from the infected vehicle group. The infected treatment groups also did not differ significantly from one another. This pattern does not support preservation of vertical exploratory activity by any treatment. Although the combination group showed a numerically smaller decline than

either monotherapy, the corresponding comparisons were not significant and therefore do not establish behavioural protection. Likewise, the greater reduction observed with artesunate should not be interpreted as drug-induced neurotoxicity. Rearing is particularly responsive to contextual and habituation effects, and a reduction may reflect diminished novelty-driven exploration rather than neurological injury [35].

Interpretation is further limited because Day 3 represented a post-infection, pre-treatment assessment rather than a true pre-infection baseline. The groups also differed in rearing activity at that assessment. Consequently, subsequent changes could reflect differences in starting values, infection-related sickness, habituation or treatment response. Experimental malaria can produce abnormalities in locomotor, exploratory and cognitive behaviour, some of which may persist after parasitological improvement [33, 36, 37]. Nevertheless, those reports were principally derived from mouse models and provide mechanistic context rather than direct confirmation of the present findings in rats. Overall, the rearing findings indicate heterogeneous behavioural changes without demonstrating treatment-related neurological recovery. The strong parasitological responses were therefore not accompanied by measurable preservation of vertical exploration during the short observation period.

Grooming showed no uniform change over time, but a significant time-by-group interaction indicated that the direction of change differed among groups. Among infected rats, artesunate increased grooming relative to infected vehicle and clindamycin, whereas clindamycin and combination therapy did not differ significantly from infected vehicle. The comparison between artesunate and combination therapy was also not statistically significant. The pattern therefore suggests an artesunate-associated behavioural response rather than a general benefit shared by all antimalarial treatments. Self-grooming is a normal maintenance behaviour, but its frequency and organisation are also influenced by stress, arousal and anxiety-related states [38, 39]. Increased or decreased grooming may therefore have biological significance, depending on the context and sequencing of the behaviour [38,39]. Because this study measured grooming frequency without assessing duration, anatomical sequence or behavioural microstructure, the artesunate-associated increase cannot be interpreted unequivocally as reduced anxiety, neurological recovery or improved well-being.

The absence of a comparable increase in the combination group is particularly informative because that group had the lowest cumulative parasitaemia burden. This dissociation indicates that the grooming response was not simply proportional to parasite suppression. Behavioural abnormalities may persist or evolve independently after parasitological control in experimental malaria [33, 37]. Alternatively, the observed increase could

reflect treatment-associated changes in arousal or behavioural organisation rather than neuroprotection.

Together, the three open-field measures do not demonstrate a uniform behavioural advantage of artesunate, clindamycin or their combination. Artesunate produced a distinct increase in grooming but did not preserve line-crossing or rearing activity, while combination therapy achieved the lowest cumulative parasitaemia without corresponding superiority across the behavioural endpoints. The findings therefore support a dissociation between parasite clearance and short-term behavioural recovery. Given the absence of a true pre-infection behavioural baseline and the nonspecific nature of open-field measures, these results should be interpreted as treatment- and group-dependent behavioural changes rather than definitive evidence of neurological protection or injury.

Strengths and limitations

This study used a balanced eight-group design with complete observations for all 64 animals. We confirmed infection before treatment, monitored parasitaemia from Days 3–8, and estimated cumulative parasite burden using the area under the curve. We applied robust, non-parametric procedures when distributional assumptions were not met. The sample size of eight animals per group limits the precision of some behavioural comparisons. Day 3 testing occurred after infection but before treatment and was therefore not a true pre-infection baseline. Consequently, repeated measures and change-score analyses characterised behavioural trajectories but could not eliminate the influence of initial group differences. Open-field measures are also affected by habituation, motivation, anxiety and systemic illness and are not specific measures of neurological recovery [25, 29, 31, 32]. Microscopic clearance did not exclude submicroscopic parasitaemia or establish sterile cure [12, 28]. Follow-up ended on Day 8, preventing assessment of recrudescence, survival differences and long-term behavioural recovery. Both sexes were included, but sex-specific effects were not analysed

Conclusion

Within the conditions of this study, artesunate, clindamycin and their combination produced substantial antimalarial effects in *Plasmodium berghei* ANKA-infected Wistar rats. Artesunate achieved the earliest microscopic parasite clearance by Day 6, whereas clindamycin and the combination achieved clearance by Day 7. Although the combination produced the lowest cumulative parasitaemia, all three active treatments significantly reduced parasite burden relative to infected vehicle-treated animals. Behavioural responses were less consistent than the parasitological effects. Artesunate selectively improved grooming but did not prevent reductions in line crossing or rearing. The combination did not demonstrate consistent behavioural superiority over either monotherapy. Treatment-

period body-weight changes also differed among groups but did not establish a uniformly superior weight-related effect of the combination.

These findings indicate that artesunate provided the most rapid parasite clearance, while the artesunate–clindamycin combination reduced cumulative parasite exposure. However, the results do not establish pharmacological synergy, neurological recovery or neuroprotection. Longer-duration studies incorporating post-treatment parasite monitoring, validated neurological assessments, and neuropathological confirmation are required to determine the durability and neurological relevance of these treatment effects.

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Author Contributions

Conceptualisation: AAO. Methodology: OOJ, OAY, AAO & AGO Investigation: AAO, AAO, AGO Data curation: OBM, MOT & AAO. Formal analysis: ABD, AAO & OBM Writing original draft: AAO, OBM & MOT Editing: OAY, OOJ, AAO. All authors reviewed and approved the final manuscript.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request, subject to applicable institutional and ethical requirements.

Declaration Of Conflict Of Interest

The authors declare no conflict of interest.

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