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Original Research Article

Antiplasmodial and immunomodulatory effects of *Kalanchoe brasiliensis* extract in *Plasmodium berghei-infected* rats

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Abstract

Purpose: *To determine the effect of Kalanchoe brasiliensis on malaria infection and immune responses in infected rats.*

Method: *Using a randomized design, eight Wistar rats were assigned to each of the six groups in the experiment. Wistar rats were infected intraperitoneally with 10^7 malaria-parasitized red blood cells containing Plasmodium berghei. Three different treatment groups of Wistar rats were given different doses of Kalanchoe brasiliensis plant extract, which were 125, 250, and 500 mg/kg body weight, respectively; and the fourth group was given an antimalarial drug, Chloroquine. No treatment was given to the fifth infected group. The uninfected sixth group was not given any treatment. Parasitemia was monitored for four days at 24-hour intervals through blood smears and microscopy, while serum cytokines such as Tumor necrosis factor-α, interleukin- 1, interleukin-10, and interferon- γ were measured on day four using the ELISA technique.*

Results: *Rats treated with the Kalanchoe brasiliensis extract doses showed a clear reduction in parasitemia ($p < 0.001$). However, none of the three Kalanchoe brasiliensis extract doses differed significantly from chloroquine ($p > 0.05$). Untreated animals exhibited elevated pro-inflammatory cytokines such as TNF-α), whereas treated groups showed statistically significantly reduced levels of this marker (TNF-α) at $P < 0.01$. In contrast, IL-10 levels increased following Kalanchoe brasiliensis extract treatment at 500 mg/kg dose in comparison with the untreated group ($P < 0.01$).*

Conclusion: *Kalanchoe brasiliensis shows promising antimalarial and immune-balancing effects. It may serve as a useful complementary option in malaria treatment.*

Keywords: *Antiplasmodial, Immunomodulatory, Kalanchoe brasiliensis, Plasmodium berghei*

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INTRODUCTION

Malaria is the most significant parasitic disease worldwide, with over 200 million cases globally and over 600,000 deaths annually [1]. Chronic malaria in humans can lead to breakdown of the immune system [2]. The various species of *Plasmodium* cause immunopathological alterations and modulate the production and action of different pro- and anti-inflammatory

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cytokines released by various cell populations during the course of infection [3]. The regulation of these immune responses can influence the clinical course of the disease [4]. The efforts made by the immune system to eliminate the *Plasmodium* parasite led to the activation of several immune cell populations and the production of important mediators such as cytokines [5]. In this sense, the balance of the production of pro-inflammatory and anti-inflammatory cytokines is essential for controlling parasitic infection and avoiding excessive tissue damage [6]. Cytokines are critical players during malaria infection because they help determine pathogen control and the severity of disease; their production profiles and balance are directly or indirectly related to pathogenesis [5]. It has been previously suggested that balanced production of pro-inflammatory (e.g., Interleukin-1 (IL-1), Tumor necrosis factor-α (TNF-α)), anti-inflammatory (e.g., Interleukin-10 (IL–10)), and regulatory (e.g., Interferon-γ) cytokines are required to achieve a mild malaria disease [7].

Although chemically synthesized drugs such as Artemisinin-based combination therapies (ACTs) remain the principal therapy, the emergence of resistance has led scientists to search for new alternatives, including medicinal plants [8]. *Kalanchoe brasiliensis Cambess* has been employed in traditional medicine for a variety of conditions, including malaria [9]. The present study aimed to evaluate the cytokine profile of rats infected with malaria and treated with the ethanolic extract from *K. brasiliensis* leaves.

METHODS

Study design

The study design used was a randomized controlled design.

Housing and feeding

Forty-eight male Wistar rats weighing between 150 - 220 g were provided from the Physiology Department animal house unit and kept in six separate plastic cages, with each cage containing eight (8) Wistar rats, which were maintained under standard laboratory conditions (25 ± 2 °C, 12-hour dark/light cycle, and humidity of 30 – 60 %). The rats were allowed to acclimatize for 7 days before treatment and fed *ad libitum* with standard feed and water.

Plant collection

The *K. brasiliensis* leaves were sourced from a residential street in Ogooluwa, Ado Ekiti, South-West, Nigeria. It was confirmed at the Department of Biology, Afe Babalola University, Ado Ekiti. The geographic coordinates for the collection region were 7° 37′ 24″ N, 5° 13′ 15″ E. *K. brasiliensis* leaves that were collected were washed under running water to remove soil debris. They were then shade-dried and ground into a powder form. It was stored in an airtight container for extraction.

Kalanchoe brasiliensis extraction procedure using Soxhlet apparatus

Thirty grams of the powdered leaves were weighed. The weighed powdered samples were inserted into a thimble composed of filter paper. Ethanol was put into a round-bottom flask that could hold 500 mL of the solvent. The samples were placed in a thimble and then positioned inside the Soxhlet extractor. The Soxhlet extractor was connected to the round-bottom flask. The reflux condenser was properly mounted above the Soxhlet extractor. The whole apparatus was placed on a hot burner. The solvent was slowly heated until it boiled. Solvent vaporized through the side arm and condensed in the condenser. The condensed solvent dripped into the Soxhlet chamber and extracted the soluble compound from the sample. When the solvent reached the siphon arm, the extract was siphoned back into the round-bottom flask. This cycle was repeated automatically.

Parasitemia determination of donor mice

The donor mice (four) were obtained from the Institute of Advanced Medical Research and Training (IMRAT), University of Ibadan, where they were inoculated with *Plasmodium berghei* (NK-65 strain) for experimental use only. The donor mice were inoculated with 10^7 parasitized red blood cells, which were contained in a 200 µL volume. The donor mice were inoculated intraperitoneally with 200µL *Plasmodium berghei*-infected blood and were allowed to attain a 25% parasitemia level over a period of four days post-infection, which was measured daily by collecting blood samples from the tail end, and smears stained with 10 % Giemsa stain were made on clean glass slides for microscopic confirmation [10].

For calculation, the average mouse Red Blood Cell (RBC) count is 8×10^9Red Blood Cell/mL. Parasitized Red Blood Cell/mL = Red Blood Cell count × Parasitemia/100 …… (1)

Parasitemia (%) = (PRBC/Total RBCs counted) 100 (2)

Therefore, $8\times10^9 \times 25\ \% = 2\times10^9$ PRBC/mL

Volume needed for 1×10^7 PRBC = $1x10^7/2x10^9$ = 0.005 mL = 5 µL

Hence, 5 µL of donor blood from a mouse contained 1×10^7 PRBC

Due to the small size of the 5 µL of inoculum, it was diluted with isotonic saline. Therefore, 5 µL of donor blood was mixed with 195 µL of sterile isotonic saline to give a final volume of 200 µL, which was injected into the donor mice intraperitoneally [10].

Parasite maintenance and transfer to Wistar rats

The donor mice were important because they serve as the animal models that keep *Plasmodium* parasites alive. Using the parasitized blood dilution method [10] as stated above, the *Plasmodium berghei* NK 65 strain was kept alive in an albino mouse that weighed 20 – 25 g. Blood was taken from the donor mouse that attained 25% parasitemia (5 µL) and mixed with physiological saline (195 µL). 0.2 mL or 200 µL of the mixture was transferred or injected intraperitoneally into the recipient Wistar rats.

Rat models

Male Wistar rats were used in this study, weighing 150 – 220 g. 0.2 mL of blood containing 1×10^7 *Plasmodium* berghei-infected red blood cells collected from donor mice that attained 25 % parasitemia was intraperitoneally injected into the rats. The animals were then monitored daily for parasitemia and other disease symptoms for four days [10].

Grouping

The animals were randomly divided into six groups containing eight Wistar rats per group. The malaria-negative control group consisted of rats infected with malaria but not receiving treatment. The uninfected group contained Wistar rats that were not infected with *Plasmodium berghei,* and no treatment was given to them. The positive control group consisted of malaria-infected rats administered chloroquine (10 mg/kg body weight) via the oral route. The three groups consisted of malaria-infected rats orally administered the extract at 125, 250, and 500 mg/kg body weight, respectively. Daily administration of the extract began four days after infection and continued for a further four days post-infection. Blood films were made on the first and fourth days of administering the chloroquine (10 mg/kg body weight) and extracts (different dosages) so that the number of blood parasites could be counted.

Giemsa staining

Blood samples were collected from the tail end of the infected rats without anesthesia. Blood smears were prepared by placing one drop of blood on a clean glass slide. The smear was allowed to air-dry, and it was fixed in methanol for about 10 minutes. The slides were stained in Giemsa solution diluted 1:10 with phosphate-buffered saline for 10 minutes. They were rinsed with water and examined under a light microscope.

Cytokine assays using enzyme-linked immunosorbent assay techniques

Cytokine levels were determined in rat serum samples using a commercial ELISA kit purchased from Mornmed Medical (Nanjing, Jiangsu, China). The manufacturer's procedure was followed.

Blood sample collection

Blood was collected four days post-infection of the male Wistar rats through retro-orbital sinus puncture with heparinized glass capillaries. Blood was pooled in a serum-separating tube. It was centrifuged at 1500 RPM for 10 min. Serum samples were extracted and stored at −80 °C before use. Serum samples were thawed and diluted 1:10, and the absorbance readings were compared to a serially diluted standard curve. Duplicate measurements for each sample and control were averaged, and background was corrected using the zero standard. Concentrations of the samples were calculated from the log–log standard curve and adjusted for the dilution factor. Optical density readings were taken at 450 nm within 15 minutes using an ELISA reader (Techan, China).

Data analysis

Statistical evaluations were visualized through one-way analysis of variance (ANOVA), followed by Pearson correlation analysis. Statistically significant effects were interpreted at a threshold of $P < 0.05$. Graphical representations were created using GraphPad Prism software (version 9.0 for Mac OS; GraphPad Software, San Diego, California, USA). Tukey's post hoc test was used in this study.

RESULTS

Day 4 parasitemia and pairwise comparisons of *Kalanchoe brasiliensis* extracts with untreated and chloroquine-treated groups were recorded in Table 1. Comparison of the level of TNF-α on day 4 was carried out among all groups (Figure 2);

there was a statistically significant decrease in TNF-α in all three doses of *Kalanchoe brasiliensis* extracts ($p < 0.05$) compared with the untreated group. In Figure 3, there was a decrease in interleukin–1 level in all three *Kalanchoe brasiliensis* extract groups, but the difference was not statistically significant between all the *Kalanchoe brasiliensis* extract groups and the untreated group ($p > 0.05$). Interleukin–10 increased only in *Kalanchoe brasiliensis* extracts (500 mg/kg), and the increase was statistically significant ($p < 0.01$) compared with the untreated group (Figure 4). Interferon– γ levels were decreased in all three treatment groups of *Kalanchoe brasiliensis* extracts, but the decrease was not statistically significant at $p > 0.05$ (Figure 5).

Table 1: Day 4 parasitemia and pairwise comparisons of *Kalanchoe brasiliensis* extracts with untreated and chloroquine-treated groups (n=8)

Group	Dose (mg/kg)	Parasitemia (%; mean ± SD)	*P*–value vs untreated	*P*–value vs chloroquine
Kalanchoe brasiliensis	125	0.13 ± 0.10	1.63×10^{-8}	0.9713
Kalanchoe brasiliensis	250	1.68 ± 1.16	1.16×10^{-7}	0.7997
Kalanchoe brasiliensis	500	0.08 ± 0.10	1.54×10^{-8}	0.9610
Chloroquine	10	0.68 ± 0.97	1.34×10^{-8}	
Untreated infected	-	11.65 ± 2.41		

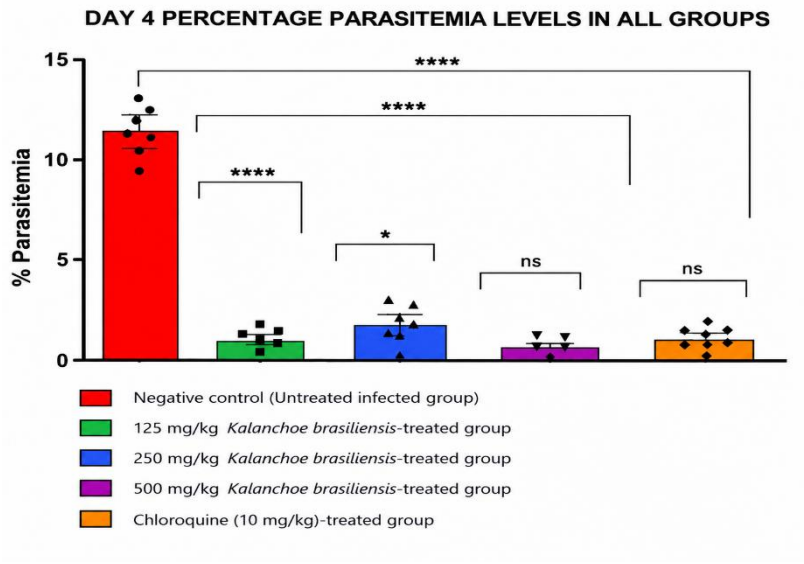

Figure 1: Comparison of the effect of treatments on parasitemia on day 4 among all groups (n = 8). ns- not significant ($P > 0.05$); **- $P < 0.01$; ***- $P < 0.001$; ****- $P < 0.0001$

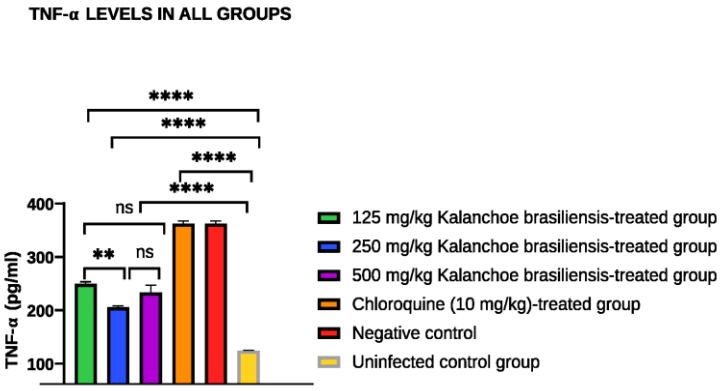

Figure 2: Comparison of the level of TNF-α on day 4 among all groups at $P < 0.05$. Where ns - not significant ($P > 0.05$); ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$

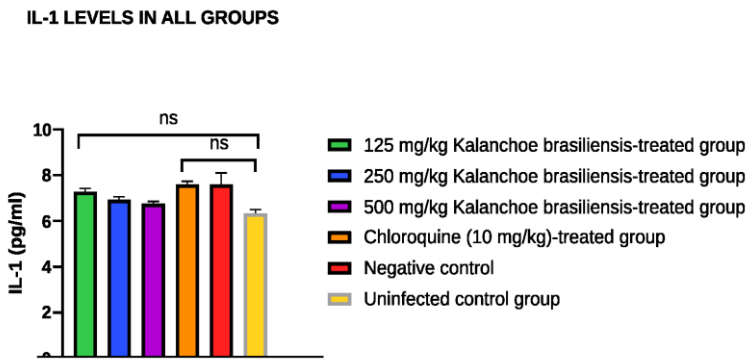

Figure 3: Comparison of the levels of IL-1 on day 4 parasitemia levels among all groups at $p < 0.05$. Where ns - not significant (P> 0.05); ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$

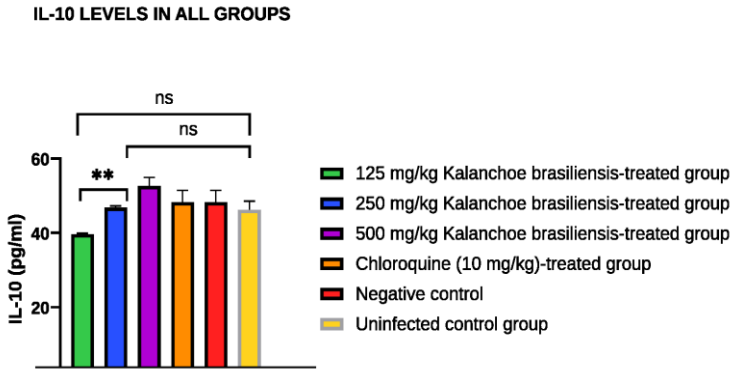

Figure 4: Comparison of the levels of IL-10 on day 4 parasitemia levels among all groups at $P < 0.05$. Where ns - not significant (P > 0.05); ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$

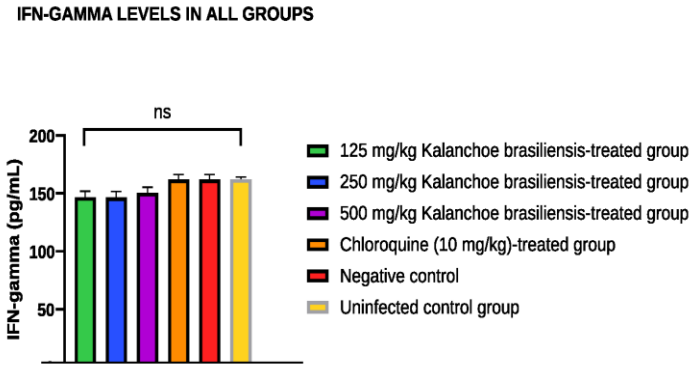

Figure 5: Comparison of the levels of IFN - - γ on day 4 parasitemia levels among all groups at $P < 0.05$. Where ns - not significant (P > 0.05); ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$

DISCUSSION

The findings from this study showed a significant decrease at $P < 0.001$ in blood parasite level in the group treated with the *Kalanchoe brasiliensis* extract on Day 4, compared with the untreated malaria group. The 500 mg/kg and 125 mg/kg doses of *Kalanchoe brasiliensis* produced the highest malaria parasite clearance, respectively, while the 250 mg/kg dose of *Kalanchoe brasiliensis* extract, although effective, showed a comparatively lower effect. This response was therefore not strictly dose-dependent. The extract's anti-plasmodial activities may be attributed to the presence or accumulation of some bioactive phytochemicals, including phenolics and flavonoids, which have been previously proven to interfere with parasite survival and growth [11].

The level of blood parasite decrease seen in this study agrees with White and his colleagues that

plant-based treatments of malaria infections can disrupt *Plasmodium* parasite metabolism and halt its replication within the red blood cells [12]. Chloroquine therapy showed a numerical parasite reduction; however, the higher dose of *Kalanchoe brasiliensis* extract (500 mg/kg) compared favorably. Statistically, there was no difference among the three doses of *Kalanchoe brasiliensis* extract (125, 250, and 500 mg/kg) compared with chloroquine ($P > 0.05$). This indicated that the parasitemia levels following extract treatment were statistically comparable with those observed following chloroquine treatment, which proved that *Kalanchoe brasiliensis* extract has good therapeutic properties.

Apart from a decrease in the blood *Plasmodium* parasite, the present study also showed changes in pro-inflammatory and anti-inflammatory cytokines. In the untreated negative group, IL-1 did not differ significantly between the untreated infected control and the 125 mg/kg *Kalanchoe brasiliensis* treated group ($P > 0.05$). Similarly, no significant difference was observed between the untreated infected control and the chloroquine antimalarial drug group ($P > 0.05$). TNF-α was also increased in the untreated group ($P < 0.001$), and IFN-γ was not significantly different from any treatment group ($P > 0.05$). This result supported the claim that cytokines play a major role in malaria pathogenesis, and increased release of these cytokines contributes to fever, tissue damage, and disease severity [13]. IFN-γ helps to stimulate macrophages, which helps in *Plasmodium* blood clearance, but increased IFN-γ release can influence worsening immunopathology [14]. *Kalanchoe brasiliensis* extract-treated groups markedly reduced the level of TNF-α, $P < 0.001$ compared with the untreated group, and in IFN-γ, there were numerical variations, but not statistical significance, $P > 0.05$ compared with the untreated group. IL-1 had numerical variations in the treatment groups but was not statistically significant compared with the untreated group. The reduction in TNF-α seen after treatment with *Kalanchoe brasiliensis* extracts showed suppression of the elevated inflammatory response, which assisted in getting a good clinical outcome and preventing tissue damage [13].

Furthermore, IL-10, which is an important anti-inflammatory cytokine, increased in the group treated with *Kalanchoe brasiliensis* extract (500 mg/kg) when compared with the untreated negative control group ($p < 0.01$). There was no statistical difference between the 125 mg/kg and 250 mg/kg *Kalanchoe brasiliensis-treated* groups compared with the untreated group ($p > 0.05$. Anti-inflammatory cytokines are very important in reducing inflammatory responses and also help in controlling immune homeostasis during malaria infections. Therefore, increased IL-10 release suggests that *Kalanchoe brasiliensis* promotes anti-inflammatory responses, especially at higher doses (500 mg/kg), which in turn counter the harmful immune responses driven by pro-inflammatory cytokines [15].

This study found a relationship between parasitemia and cytokine levels. The untreated negative group had the highest level of parasitemia. The untreated negative group also had the highest level of TNF-α, IL-1, and IFN-γ. This result showed that increased malaria parasites in the blood stimulated inflammatory responses during malaria infection. It was also noted that the malaria-treated groups exhibited decreased parasitemia as well as a moderate reduction in cytokine levels, which indicated that the *Kalanchoe brasiliensis* extract inhibits parasite multiplication and controls the immune response [12].

The marked reduction in parasitemia after treatment with *Kalanchoe brasiliensis* extract was accompanied by alterations in the cytokine profile, particularly TNF-α. This finding is biologically relevant because the host inflammatory response plays a dual role in malaria, contributing to parasite control while excessive inflammatory activation may promote immunopathology. TNF–α and IFN–γ participate in mechanisms involved in parasite clearance, whereas IL-10 functions as an important regulatory cytokine that limits excessive inflammation. Thus, effective antimalarial therapy may involve not only reductions of parasite burdens but also restoration of an appropriate balance between pro-inflammatory and regulatory responses [16].

CONCLUSION

The outcome of this study shows that the ethanol extract of *Kalanchoe brasiliensis* leaves has anti-plasmodial and immunomodulatory properties. *Kalanchoe brasiliensis* leaves reduce parasitemia levels and, at the same time, the highest dose of *Kalanchoe brasiliensis* extract modulates pro-inflammatory and anti-inflammatory cytokines, making it suitable for malaria treatment. There is a need for further study to determine the plant toxicity level and extend the post-infection period/duration beyond four days.

DECLARATIONS

Acknowledgement/Funding

None.

Ethical approval

The study was approved by the Health Research and Ethics Committee (HREC), Afe Babalola University, Ado Ekiti (Approved Number: ABUADHREC/21/01/2026/841).

Use of Artificial intelligence/Large language models

We also declare that we did not use generative artificial intelligence (AI) and AI-assisted technologies in writing this manuscript.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the six authors named in this article, and the authors will bear all liabilities related to claims in the content of this article.

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