Pharmacogn J. 2025; 17(1): 84-88
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Research Article

# The Mechanism of Nanocurcumin in Inhibiting Parasitemia in *Plasmodium berghei ANKA* (PbA) Model Mice

**Risma[1,2], Aryati[3]*, Bambang Purwanto[4]**

## ABSTRACT

*Plasmodium falciparum* is the cause of malaria falciparum, the most severe type of malaria, and the only malaria parasite that can cause complications such as microvascular disease, cerebral malaria, severe anemia, shock, acute renal failure, and shortness of breath. In Southeast Asia, Indonesia has the highest incidence of malaria. The WHO estimated that in 2019, there were 658,380 malaria cases and 1,170 malaria-related deaths. Curcumin (Curcuma longa) is a spice that has been used in Southeast Asia for centuries. It contains the active ingredient curcumin (bis-α, β-unsaturated β-diketone), which has antioxidant, anti-inflammatory, hepatoprotective, and antimalarial properties. However, curcumin has low water solubility and very limited bioavailability. By examining the observed phenomenon, it is possible to investigate how nanocurcumin might impact parasitemia levels in *P. berghei ANKA* model mice. This research involved 36 female BALB/c mice aged 7–10 weeks, divided into four groups, all of which were infected with *P. berghei ANKA*. After infection, the groups were treated for 2 weeks as follows: the control group (no nanocurcumin administered), treatment group I (50 mg/kg body weight [kgbw]), treatment group II (100 mg/kgbw), and treatment group III (150 mg/kgbw). The results indicated a significant difference among groups ($p < 0.05$, 0.036). The conclusion of this experiment is that administering nanocurcumin to mice infected with the *Plasmodium* parasite significantly reduces parasitemia levels in the blood, particularly at a dose of 150 mg/kgbw.

**Keywords:** Parasitemia, *Plasmodium berghei ANKA*, Nanocurcumin.

**Risma[1,2], Aryati[3]*, Bambang Purwanto[4]**

[1]*Doctoral Program of Medical Science, Faculty of Medicine, Universitas Airlangga, Surabaya, INDONESIA.*

[2]*Department of Parasitology, Faculty of Medicine, Hang Tuah University, Surabaya, INDONESIA.*

[3]*Department of Clinical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, INDONESIA.*

[4]*Department of Medical Physiology and Biochemistry, Faculty of Medicine, Universitas Airlangga, Surabaya, INDONESIA.*

**Correspondence**

**Aryati**

Department of Clinical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, INDONESIA.

E-mail: aryati@fk.unair.ac.id

**History**

- Submission Date: 12-12-2024;
- Review completed: 02-01-2025;
- Accepted Date: 10-01-2025.

**DOI : 10.5530/pj.2025.17.11**

**Article Available online**

http://www.phcogj.com/v17/i1



## INTRODUCTION

*Plasmodium* is a genus of parasitic organisms that cause malaria, which manifests with symptoms such as an enlarged spleen, muscle pain, nausea, abdominal pain, chills, headaches, anemia-induced weakness, and varying degrees of fever. Although *Plasmodium* is a fairly large genus, only a few species can infect humans: *P. malariae, P. vivax, P. ovale, P. falciparum, and P. knowlesi*.[1] Conversely, certain professionals argue that malaria is a short-term or long-lasting illness caused by *Plasmodium* infection. The parasite infects red blood cells, producing symptoms such as fever/chills, weakness, anemia (blood loss), and spleen enlargement. *Plasmodium falciparum* is the cause of malaria falciparum, the most severe type of malaria, and the only malaria parasite that can cause complications such as microvascular disease, cerebral malaria, severe anemia, shock, acute renal failure, and shortness of breath.[2]

Among all *Plasmodium* species, *P. falciparum* has the shortest infective period but causes the highest parasitemia levels. Its gametocytes develop 8 to 15 days after the parasite enters the bloodstream. The infestation caused by *P. falciparum* can spread to various organs, including the brain, kidneys, lungs, liver, and heart, leading to severe malaria and complications.[3] When *P. falciparum* parasites persist in tissues—especially in the brain—this phenomenon is referred to as sequestration. In severe malaria cases, parasites are often undetectable in peripheral blood due to sequestration.[4]

In Southeast Asia, Indonesia has the highest incidence of malaria. The WHO estimated that in 2019, there were 658,380 malaria cases and 1,170 malaria-related deaths. Despite efforts to eliminate malaria, Indonesia, like many other Southeast Asian countries, faces significant challenges due to the presence of multiple infection species and the difficulty of identifying specific transmission hotspots. By 2017, most districts in Indonesia were declared "malaria-free." However, Indonesia's diverse population, spread across more than 5,000 islands and numbering over 260 million, presents unique challenges, including internal migration, socioeconomic inequalities, and decentralized administration.[5]

Medicinal plants have the potential to modulate immune responses and produce therapeutic effects. In India, for example, many people consume foods containing herbs and spices like garlic, ginger, curcumin, and black pepper, which are believed to combat malaria effectively.[6] Curcumin (Curcuma longa), a spice used in Southeast Asia for centuries, is well known for its coloring and flavoring properties. It is one of the most affordable spices globally and has been used for 4,000 years to treat various diseases. Curcumin contains the active ingredient curcumin (bis-α, β-unsaturated β-diketone), which exhibits antioxidant, anti-inflammatory, antibacterial, anticarcinogenic, hepatoprotective, and nephroprotective properties, as well as antithrombotic, cardioprotective, hypoglycemic, and antirheumatic effects. Curcumin has also demonstrated antimalarial activity in vitro against several *Plasmodium* species.[7]

Curcumin is the primary yellow pigment derived

**Cite this article:** Risma, Aryati, Purwanto B. The Mechanism of Nanocurcumin in Inhibiting Parasitemia in *Plasmodium berghei ANKA* (PbA) Model Mice. Pharmacogn J. 2025;17(1): 84-88.

from the rhizomes of the Zingiberaceae family. Numerous studies have shown that curcumin possesses anti-inflammatory, antioxidant, and pharmacological properties while also acting as an immunomodulator, promoting wound healing, neuroprotection, and anti-aging. However, despite its high antioxidant activity, curcumin has poor water solubility and extremely low bioavailability. To address this issue, nanoparticle technology has been developed to improve the delivery of drugs with poor bioavailability. In terminology, nanoparticles are structures with dimensions ranging from 1 to 100 nm. Over the past decade, nanomaterials have undergone identification and development through a multidisciplinary and interdisciplinary approach involving various nanotechnology methods. Nanocurcumin, a nanotechnology-based curcumin formulation, enhances curcumin's solubility and bioavailability, addressing its inherent limitations. Given these advancements, this study aims to investigate the effects of nanocurcumin on parasitemia levels in BALB/c mice infected with the *Plasmodium berghei ANKA* model.[8]

## METHODS

This research is an experimental in vivo study that utilized a randomized control group design and performed testing only once at the end of the study. The study involved 36 female BALB/c mice, aged 7–10 weeks, obtained from PUSVETMA Surabaya. The experiment was conducted at the Integrated Biomolecular Hyperbaric Laboratory, Faculty of Medicine, Hang Tuah University, and was approved by the Ethics Committee (No. 2.KEH.046.04.2024). The mice were divided into four groups and randomly assigned as follows:

1. Control group (K1): Mice that were not given nanocurcumin but were infected with *Plasmodium ANKA*.
2. Treatment group I (K2): Mice that were given nanocurcumin at a dose of 50 milligrams per kilogram of body weight (mg/kgbw) for 2 weeks and were infected with *Plasmodium ANKA*.
3. Treatment group II (K3): Mice that were given nanocurcumin at a dose of 100 mg/kgbw for 2 weeks and were infected with *Plasmodium ANKA*.
4. Treatment group III (K4): Mice that were given nanocurcumin at a dose of 150 mg/kgbw for 2 weeks and were infected with *Plasmodium ANKA*.

### Nanocurcumin preparation

The nanocurcumin material used was an isolate of curcumin.

### Donor mice preparation

Donor mice were prepared using five mice, each of which was infected with 200 µL of frozen blood containing *Plasmodium ANKA*. When the parasitemia level in each mouse exceeded 20%, the mice were terminated. Blood was then collected via cardiac puncture and used to infect the experimental groups intraperitoneally. Each mouse in the experimental group was infected with 0.2 mL of blood containing $1 \times 10^7$ infected erythrocytes.

### Parasitemia calculation

Parasitemia was calculated using the thin smear method with Giemsa staining. The degree of parasitemia in infected mice was examined daily from day 1 to day 12 post-*Plasmodium ANKA* infection. The calculation was performed using the following formula:

$$\frac{\text{number of infected erythrocytes}}{\text{1000 erythrocytes}} \times 100\%$$

### Statistical analysis

Parasitemia data were collected on day 12 for each mouse and then compared across groups using the Shapiro-Wilk normality test. The Shapiro-Wilk test was chosen due to the sample size being fewer than 50. If the data were normally distributed, a homogeneity test was performed. In cases where homogeneity was confirmed, parametric testing was conducted using one-way ANOVA to compare group means.

## RESULTS

The experiment was conducted for approximately 2 weeks, with parasitemia monitored daily from day 1 to day 12. On day 12, parasitemia data were collected for each mouse, yielding the following results:

The table above presents data for the control group (K1) and treatment groups (K2, K3, and K4), each consisting of 7 mice. Initially, there were nine mice per group, but some became ill and died before the designated time. In group K1, parasitemia ranged from 21.20% to 41.20%, with an average of 28.82%. In group K2, parasitemia ranged from 15.00% to 33.60%, with an average of 27.02%. In group K3, parasitemia ranged from 3.70% to 31.00%, with an average of 18.31%. In group K4, parasitemia ranged from 1.20% to 39.90%, with an average of 15.44%. Subsequently, a normality test was conducted using the Shapiro-Wilk method, producing the following results:

The Shapiro-Wilk test results indicated $p > 0.05$ for all groups, suggesting that the data were normally distributed. A homogeneity test was then performed to determine whether the variances were homogeneous, as this would influence the selection of parametric or

**Table 1. Data distribution.**

| PARASITEMIA | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Group** | **Mean** | **Median** | **Variance** | **Std. Deviation** | **Min** | **Max** | **Frequency** |
| **K1** | 28.82 | 27.10 | 51.53 | 7.17 | 21.20 | 41.20 | 7 |
| **K2** | 27.02 | 28.10 | 38.55 | 6.20 | 15.00 | 33.60 | 7 |
| **K3** | 18.31 | 16.20 | 82.04 | 9.05 | 3.70 | 31.00 | 7 |
| **K4** | 15.44 | 14.10 | 184.95 | 13.59 | 1.20 | 39.90 | 7 |
| **Total** | | | | | | | **28** |

**Table 2. Normality test results.**

| NORMALITY TEST | |
|---|---|
| **Group** | **Significance** |
| **K1** | 0.493 |
| **K2** | 0.260 |
| **K3** | 0.824 |
| **K4** | 0.478 |

**Table 3. Homogeneity test results.**

| HOMOGENEITY TEST | |
|---|---|
| | **Significance** |
| **Parasitemia** | 0.208 |

**Table 4. Parametric statistical test results using one-way ANOVA.**

| PARAMETRIC TEST | |
|---|---|
| | **Significance** |
| **Parasitemia** | 0.036 |

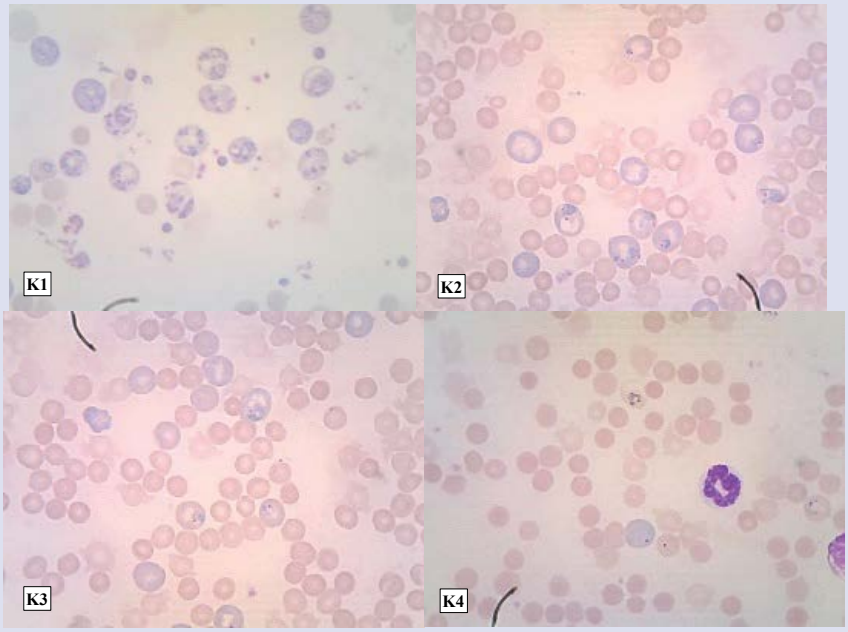


**Figure 1.** Microscopic images obtained using 100× magnification. K1 represents the control group (not given nanocurcumin but infected with *Plasmodium ANKA*), K2 represents treatment group I (given nanocurcumin at a dose of 50 mg/kgbw for 2 weeks and infected with *Plasmodium ANKA*), K3 represents treatment group II (given nanocurcumin at a dose of 100 mg/kgbw for 2 weeks and infected with *Plasmodium ANKA*), and K4 represents treatment group III (given nanocurcumin at a dose of 150 mg/kgbw for 2 weeks and infected with *Plasmodium ANKA*).

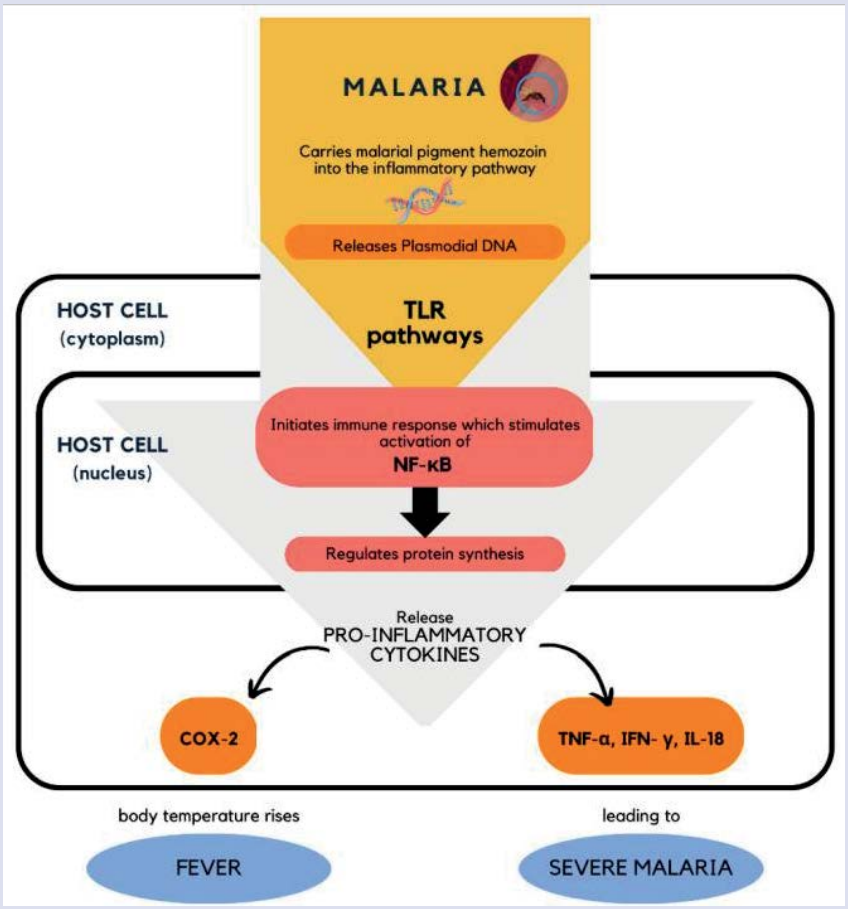


**Figure 2.** Cellular-level mechanisms of action of malaria infection on host cells.[9]

non-parametric statistical tests. The homogeneity results are presented in the following table.

The homogeneity test results demonstrated $p > 0.05$ (0.208), indicating that the data were homogeneous. Based on this finding, a parametric statistical test (one-way ANOVA) was performed to compare the means across the groups and determine whether significant differences existed. The following table presents the results of the parametric test.

The one-way ANOVA results demonstrated $p < 0.05$ (0.036), confirming that the research data were statistically significant and that there were differences among the experimental groups.

## DISCUSSION

The research results presented in Table 1 demonstrate the data distribution, with considerable variation across the groups, particularly in K3 and K4. This suggests that a greater variance indicates data points are spread further from the average, while a lower variance implies that the data points are closer to the average. Observing the lowest parasitemia values, there is a gradual decrease from the control group (K1) to the treatment groups (K2, K3, and K4). However, the highest parasitemia value in group K4 is greater than those in K2 and K3, although still below the highest value in the control group. These variations may be attributed to several factors, such as human error during nanocurcumin administration, inconsistencies in blood infection procedures, or changes in the mice's health (e.g., adjustment disorders or stress), particularly at the beginning of treatment.

Curcumin is the primary yellow pigment derived from the rhizomes of the Zingiberaceae family. Its potential as an antimalarial therapy has been extensively studied, especially in vitro, where significant results have been observed when combined with antimalarial drugs. Curcumin has demonstrated the ability to reduce inflammatory mediators such as IL-8, NF-kB, IL-1β, IL-6, TNF-α, and reactive oxygen species. Malaria is one of the diseases that disrupt the inflammatory response through signaling pathways involving Toll-like receptors (TLRs), which are cell surface receptors. When *Plasmodium* DNA circulates or is released, it triggers TLRs to initiate inflammatory responses, including the release of TNF-α, IL-6, COX-2, IL-10, IL-1, and IL-8. Elevated inflammatory factors result in malaria symptoms such as fever, and if left untreated, severe malaria can develop.[9]

Tumor necrosis factor-alpha (TNF-α) is a major pro-inflammatory cytokine produced by macrophages and monocytes, playing a crucial role in the pathogenesis of severe malaria. TNF-α is a key component of the innate immune system, causing various clinical manifestations such as fever, edema, pain, and tissue damage. It serves as the first line of defense against microorganism infections. TNF-α mobilizes inflammatory cells—particularly monocytes and neutrophils—to infection sites, activates neutrophils and macrophages to eliminate pathogens, stimulates the expression of vascular adhesion molecules for leukocytes, promotes the expression of MHC I and MHC II, increases the production of other pro-inflammatory cytokines, and induces apoptosis in inflamed cells. While macrophages are the primary source of TNF-α, it can also be produced by T cells, B cells, and mast cells.[7,10]

At low levels, TNF-α inhibits the growth of the parasite's blood stage by activating the cellular immune system, directly killing the parasite, although its efficacy is limited. A study by Cruz et al. (2016) demonstrated that TNF-α can inhibit *P. falciparum* proliferating-cell nuclear antigen-1 (PfPCNA1), a critical regulator of DNA replication and repair. Inhibiting PfPCNA1 disrupts the parasite's function, leading to its death. Conversely, excessive TNF-α levels in response to hyperparasitemia (excessive parasite growth) can cause severe and fatal tissue damage. Given curcumin's effectiveness in reducing inflammatory factors, especially TNF-α, as described above, the cellular immune system can act more efficiently to eliminate parasites.[11-15]

## CONCLUSION

This study concludes that administering nanocurcumin to mice infected with the Plasmodium parasite significantly reduces parasitemia levels in the blood, particularly at a dose of 150 milligrams per kilogram of body weight over two weeks. It is expected that optimizing the administration method can further enhance the efficacy of nanocurcumin.

## ACKNOWLEDGEMENTS

The authors would like to express their gratitude to the Faculty of Medicine, Universitas Airlangga, Surabaya, and the Departement of Parasitology, Faculty of Medicine, Hang Tuah University, Surabaya, for their support during this study.

## ETHICAL CONSIDERATION

The study was performed at the Integrated Biomolecular Hyperbaric Laboratory, Faculty of Medicine, Hang Tuah University, and received approval from the Ethics Committee (No. 2.KEH.046.04.2024).

## CONFLICTS OF INTEREST

The authors have stated that they do not have any conflict of interest regarding this study.

## AUTHORS' CONTRIBUTION

All authors contributed to article preparation and paper revision and have collectively assumed responsibility for all aspects of this study.

## DATA AVAILABILITY

The article contains all the necessary data to support the results, and no supplementary source data is needed.

## REFERENCE

1. Escalante AA, Cepeda AS, Pacheco MA. Why Plasmodium vivax and Plasmodium falciparum are so different? A tale of two clades and their species diversities. Malar J. 2022;21(1):139.
2. Yunita NN, Tatontos EY, Urip U. Analisis Jenis Plasmodium Penyebab Malaria Terhadap Hitung Jumlah Trombosit. Jurnal Analis Medika Biosains (JAMBS). 2019;6(1):58.
3. Maier AG, Matuschewski K, Zhang M, Rug M. Plasmodium falciparum. Trends Parasitol. 2019;35(6):481–2. https://doi.org/10.1016/j.pt.2018.11.010
4. Dwiridal HP, Adrial A, Darwin E. Gambaran Histopatologi Hepar Mencit (Mus musculus Balb/C) yang Diinfeksi dengan Plasmodium berghei. Jurnal Ilmu Kesehatan Indonesia. 2022;2(2):1–8.
5. World Health Organization. World Malaria Report 2022. 1st ed. Vol. 1. 2022.
6. Veronica E, Amelia I, Yunatan KA, Chrismayanti NKSD, Mahendra AN. Potensi Kombinasi Ekstrak Daun Kelor (Moringa oliefera) dan Artemisia (Artemisia annua) Sebagai Antimalaria Plasmodium falciparum. Jurnal Ilmiah Kesehatan Sandi Husada. 2020;12(2):831–41.
7. Kingston DGI, Cassera MB. Antimalarial Natural Products. 2022. p. 1–106.
8. Ghosh A, Banerjee T, Bhandary S, Surolia A. Formulation of nanotized curcumin and demonstration of its antimalarial efficacy. Int J Nanomedicine. 2014;9:5373–87.
9. Jamil SNH, Ali AH, Feroz SR, Lam SD, Agustar HK, Mohd Abd Razak MR, et al. Curcumin and Its Derivatives as Potential Antimalarial and Anti-Inflammatory Agents: A Review on Structure–Activity Relationship and Mechanism of Action. Pharmaceuticals. 2023;16(4):609.

10. Sahebkar A, Cicero AFG, Simental-Mendía LE, Aggarwal BB, Gupta SC. Curcumin downregulates human tumor necrosis factor-α levels: A systematic review and meta-analysis ofrandomized controlled trials. Pharmacol Res. 2016;107:234–42.

11. Cruz LN, Wu Y, Ulrich H, Craig AG, Garcia CRS. Tumor necrosis factor reduces Plasmodium falciparum growth and activates calcium signaling in human malaria parasites. Biochimica et Biophysica Acta (BBA) - General Subjects. 2016;1860(7):1489–97.

12. Solihah R, Haris MS, Abror YK. Analysis of APO-B Serum Levels in BALB/C Mice Hypercholesterolemic Against Temulawak Extract (Curcuma xanthoriza Roxb). IJMLST. 2019;1(1):1-7. https://journal2.unusa.ac.id/index.php/IJMLST/article/view/799.

13. Pramana IBAI, Sanjaya IGPH, Hamid ARRH, Adnyana IMS, Dewi IGASM, Niryana W, Herawati S. Topical curcumin gel accelerates healing of II A – degree burns on male wistar rats (Rattus norvegicus). IJBS. 2023;17(2):189-97. https://ijbs-udayana.org/index.php/ijbs/article/view/475.

14. Rahman FI, Zulfa PO, Beočanin A, et al. Targeting phosphoglycerate dehydrogenase enzyme using ginger compounds to suppress thyroid cancer progression. Narra X. 2024;2(1):e112. https://doi.org/10.52225/narrax.v2i1.112.

15. Aini NS, Ansori ANM, Herdiansyah MA, Kharisma VD, Widyananda Murtadlo AAA, et al. Antimalarial Potential of Phytochemical Compounds from Garcinia atroviridis Griff ex. T. Anders Targeting Multiple Proteins of Plasmodium falciparum 3D7: An In Silico Approach. BIO Integration. 2024;5(1): e967. doi: 10.15212/bioi-2024-0075.

**Cite this article:** Risma, Aryati, Purwanto B. The Mechanism of Nanocurcumin in Inhibiting Parasitemia in Plasmodium berghei *ANKA* (PbA) Model Mice. Pharmacogn J. 2025;17(1): 84-88.