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RESEARCH ARTICLE

Annona muricata leaf water extract in combination with artemisinin-based combination therapy for increasing CCL19 levels in the treatment of severe *Plasmodium berghei* ANKA infection using swiss mice

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ABSTRACT

Cerebral malaria (CM) is a severe complication of *Plasmodium falciparum* infection, with resistance to antimalarial drugs, including artemisinin-based combination therapies(ACTs), posing a significant threat. CD4+ naive cells expressing CCR7 are known to play a protective role, as they readily migrate to secondary lymphoid tissues activated by CCL19 chemokines. In an effort to address this challenge, we investigated the impact of *Annona muricata*, an herbaceous and immunomodulatory plant, on CCL19 concentration. We conducted experiments on 24 mice, dividing them into four groups: one control group and three treatment groups. Groups 1 and 3 received a daily dose of 4.68 mg of *Annona muricata* leaf water extract (AM) for seven days before CM infection. After seven days of treatment, all groups were infected with CM. Groups 1 and 3 continued with a 9.36 mg AM dosage for an additional seven days, while groups 2 and 3 received a 0.819 mg ACTs dosage on the fourth day post-infection. We analyzed the CCL19 content in the mice's spleens and evaluated the data using Bonferroni post hoc, Kruskal–Wallis, and Mann–Whitney U tests. Our results demonstrated that the CCL19 levels in the AM–ACTs group significantly surpassed those in both the control group ($p = 0.009$) and the ACTs group ($p = 0.002$). This suggests that the combination of AM and ACTs led to a notable increase in CCL19 levels in Swiss mice afflicted with CM. While further research is necessary to validate and expand upon these findings, our study highlights the potential of AM to enhance CCL19 production, potentially facilitating the migration of naive T cells and reinforcing the immune response.

Keywords: *Annona muricata*; *Artemisinin-based Combination Therapy*; CCL19; Cerebral Malaria; *Plasmodium berghei*.

INTRODUCTION

Cerebral malaria is a prevalent and potentially fatal complication of *Plasmodium falciparum* infection (Sousa *et al*., 2018). Survivors of CM may also suffer from long-term cognitive and physical impairments. The immune response generated during a malaria infection significantly influences recovery and the development of severe malaria. Several studies have underscored the critical roles played by CD4+ T helper (Th) cells, CD8+ cytotoxic T (Tc) cells, and the spleen in malaria infection. Notably, CD8 T cells are instrumental in mediating protective immunity against Plasmodium liver-stage infection, and CD4 T cells also play an important role in this regard (Blanc *et al*., 2015; Urban *et al*., 2017).

On the other hand, numerous studies have established the protective role of cytokines, such as IL-10 and IL-27, against experimental cerebral malaria (ECM) (Sukhbaatar *et al*., 2020). Furthermore, the protective role of naive CD4+ Th cells in preventing the development of ECM has been validated through adoptive transfer treatment of these cells to mice susceptible to experimental cerebral malaria, induced by *Plasmodium berghei* ANKA (PbA) infection (Blanc *et al*., 2015). Naive CD4+ Th cells expressing CCR7 are more readily recruited to activate secondary lymphoid tissue. This enhanced recruitment is facilitated by the chemokine CCL19, which binds to CCR7.

The current literature suggests that CCR7 is a critical element for immune cell trafficking and recirculation, particularly lymph node homing (Förster & Rot, 2008). Naive CD8+ T cells are less likely to develop into memory T cells that express CCR7, whereas naive CD4+ Th cells expressing CCR7 are more readily recruited to activate secondary lymphoid tissue (Noor & Wilson, 2012). This distinction is likely because CCL19 and CCL21, which are the sole ligands for CCR7, play a key role in the homing of various subsets of immune cells (Alrumaihi, 2022). As a result, it can be inferred that naive CD4+ Th cells expressing CCR7 are more likely to be recruited to activate secondary lymphoid tissue compared to other immune cell subsets that do not express CCR7.

CCL19 and CCL21 are chemokines that specifically bind to CCR7, a G-protein-coupled receptor expressed by various immune cell subsets, including naive CD4+ Th cells (Lewandowski *et al*., 2022). This binding leads to the recruitment of CCR7-expressing cells to

secondary lymphoid tissue, where immune responses are initiated. In contrast, naive CD8+ T cells are less likely to express CCR7, making them less prone to be recruited for lymph node homing and secondary lymphoid tissue activation (Hauser & Legler, 2016). In summary, CCR7 and its ligands, CCL19 and CCL21, play a vital role in directing immune cell trafficking and recirculation, with naive CD4+ Th cells expressing CCR7 being particularly prone to recruitment for activating secondary lymphoid tissue compared to other immune cell subsets that lack CCR7 expression.

Immunomodulators capable of increasing the production of CCL19 by spleen cells are expected to provide a protective effect in ECM-susceptible mice during severe PbA infection. While CCL19 has been studied in various disease, such as allergic rhinitis (Zou *et al*., 2016)and cancer (Xu *et al*., 2017), its role in malaria has been largely unexplored. Notably, there have been no prior studies related to CCL19 in the context of malaria. *Annona muricata* leaf ethanol extract exhibited immunomodulatory effects in severe PbA infection, particularly in Swiss mice susceptible to ECM (Djamiatun *et al*., 2017). This extract led to an increase in IL-10, IL-27, and CXCL12 during severe PbA infection, highlighting its potential as a malaria treatment (Djamiatun *et al*., 2018).

In the field of malaria treatment, ACTs have long been regarded as the gold standard due to their proven efficacy. These therapies have played a crucial role in reducing the global burden of malaria, effectively lowering mortality and morbidity (Maiga *et al*., 2021). However, the emerging issue of drug resistance necessitates a combined approach, with artemisinin-based therapies playing a crucial role in preventing resistance development (Yasri & Wiwanitkit, 2021). Integrating *Annona muricata* introduces complementary mechanisms, potentially creating synergistic effects that can enhance treatment effectiveness, even if they differ from artemisinin's precise mechanisms. In summary, the combination of *Annona muricata* with artemisinin-based therapy not only explores new avenues but also upholds a proven and potent approach to malaria treatment, potentially addressing drug resistance and offering a holistic solution for severe *Plasmodium berghei* ANKA infection.

Understanding the role of CCL19 in malaria sets the stage for exploring potential treatments, and one such promising avenue is the utilization of *Annona muricata*, a herbal plant known for its anti-malarial properties (Abdillah *et al*., 2015). This research study aims to evaluate the effectiveness of the combination of *Annona muricata* leaf water extract with ACTs in increasing CCL19 levels. The study is conducted in Swiss mice with severe *Plasmodium berghei* ANKA infection, specifically focusing on its potential in the context of malaria.

MATERIALS AND METHODS

Research design

This research employs a laboratory experimental design with a Post-Test Only Randomized Control Group Design, using Swiss strain mice as the subjects of the study. Subjects were randomly assigned to different treatment groups and subsequently observed. The treatment involved administering AM doses, with the outcome measured through CCL19 level assessments.

Research sites

The maintenance of mice and laboratory examinations were conducted at the Parasitology laboratory of the Faculty of Medicine, UNDIP, as well as at Cebior, Gaki, the Anatomical Pathology laboratory of Diponegoro National Hospital, and Dr. Kariadi Hospital.

Population and Sample

The population for this study comprised Swiss mice aged approximately 6-8 weeks. The sample size was determined following WHO guidelines, with a minimum of 5 mice in each group. Additionally, one mouse was added as a reserve for each treatment group, resulting in a total sample size of 24 mice.

Inclusion, Exclusion, and Drop Out Criteria

In this study, inclusion criteria were established to determine the eligibility of the sample, which included female mice at 8 weeks of age with a body weight ranging from 30 to 35 grams. Additionally, the inclusion criteria required mice to be in a healthy state, characterized by a good appetite, active movement, and normal anatomy.

As for drop-out criteria, any mice that unfortunately perished during the course of the study were considered drop-outs.

The research variables were categorized as follows: the independent variable encompassed the administration of soursop leaf water extract (AM) and ACTs, while the dependent variable was the measurement of CCL19 levels.

To ensure the validity of the study, controlled variables were upheld, including factors such as age, gender, weight, food health, and environmental conditions. These measures aimed to minimize potential confounding variables and maintain the consistency and reliability of the research outcomes.

Operational definition

Table 1. Operational Definition

No	Variable	Operational definition	Scale
1	ACT (independent variable)	Anti-malarial drugs used for *P. falciparum*. The dose given is 3 mg dihydroartemisinin and 24 mg/kg BW	Nominal
2	*A. muricata* (independent variable)	The leaves of *A. muricata* were extracted using water. *A. muricata* was administered orally using an endogastric tube at a dose of 4.68 mg/day (before PbA inoculation) then 9.36 mg/day for curative (after PbA inoculation).	Nominal
3	CCL19 (dependent variable)	CCL19 is a chemokine produced by the DC/splenic macrophage ratio. CCL19 functions to recruit naive T cells. CCL19 levels were measured from the LPS-stimulated spleen cell culture supernatant using the ELISA method.	Ratio

Materials and tools

In the execution of this study, a range of materials and tools were employed. The primary material used was AM, procured from PT. Sido Muncul™. The test animals, essential for the research, were Swiss mice aged 8 weeks and weighing between 30-35 grams. These mice were sourced from a certified private experimental animal distributor in Bandung and were accompanied by a health certificate and strain confirmation from the Ministry of Agriculture.

For the donor mice, *P. berghei* ANKA was inoculated into healthy mice, and these mice were obtained from the Parasitology Laboratory, Faculty of Medicine, Gadjah Mada university. The maintenance of the mice followed the AIN-93M standard, and it necessitated the use of suitable mouse cages and designated food places for their well-being. When it came to the treatment of mice, specific tools like sondes for administration and scales for measurement were essential. The examination of CCL19 chemokine levels was conducted using a specialized ELISA kit designed for mice. This process required the use of additional tools, including microplates, a shaker, plate covers, an ELISA reader, and glass tubes. These tools were utilized for the examination of spleen culture supernatant using the ELISA method, ensuring the precision and accuracy of the research procedures.

Research procedure

The research procedure commenced with the careful selection of female Swiss strain mice aged 8 weeks, with body weights falling within the range of 30-35 grams. Subsequently, the mice were organized into groups and placed into cages, with each cage housing five mice. To ensure the consistency of environmental conditions, lighting, temperature, and humidity were maintained at identical levels for all groups. Daily care routines were diligently followed, with the cages being cleaned and the mice provided with the same standard pellet feed, readily available water, and unlimited access to both food and water.

Prior to the commencement of treatment, an acclimatization period of one week was instituted. This allowed the mice to adapt to their surroundings and ensured their readiness for the subsequent phases of the research. The acclimatization period served to mitigate stress and maintain the well-being of the experimental animals throughout the study.

PbA Inoculation

A volume of 1 ml of donor blood, obtained from a donor mouse at the Parasitology laboratory of the Faculty of Medicine, Gadjah Mada University, and containing 10^7 parasites, was intraperitoneally inoculated into an experimental mouse in a volume of 0.2 ml.

Treatment of Mice

Following an acclimatization period, the 24 mice were randomly divided into four groups using a randomized approach. These groups included one control group (K) and three treatment groups. In treatment groups, there are group 1 (P1), group 2 (P2) and group 3 (P3). The treatments administered to the mice can be seen in Figure 1.

Spleen cell culture supernatant for the measurement of CCL19 levels of DC products/splenic macrophages

For the measurement of CCL19 levels in DC products/splenic macrophages, the following steps were followed:

1. The spleen was placed in a sterile petri dish containing RPMI media.
2. The spleen was carefully extracted using tweezers, and both the media and spleen cells were collected in a conical tube.
3. The spleen cells were washed once with sterile Phosphate Buffered Saline (pH 7.2-7.4) and then centrifuged.
4. Erythrocyte lysis was performed using an erythrocyte lysing buffer.
5. The washing process was repeated twice with RPMI penstrep (*Penicillin* dan *Streptomycin*), followed by centrifugation.
6. The spleen cells were then counted and adjusted to a concentration of 3×10 million/ml. This adjustment was made by adding RPMI, 2% Fetal Bovine Serum (FBS), Penstrep, and L-Glutamine.
7. A 1 mL spleen cell suspension, containing 3×10^6 spleen cells, was dispensed into the wells of a 24-well plate.
8. The supernatant was collected for CCL19 examination of DC/ macrophage products.
9. Subsequently, 10 micrograms/ml of LPS (lipopolysaccharide) was added to the cell culture, followed by adding this mixture to each well.
10. The plate was then incubated for 72 hours at 37°C in a CO_2 incubator.

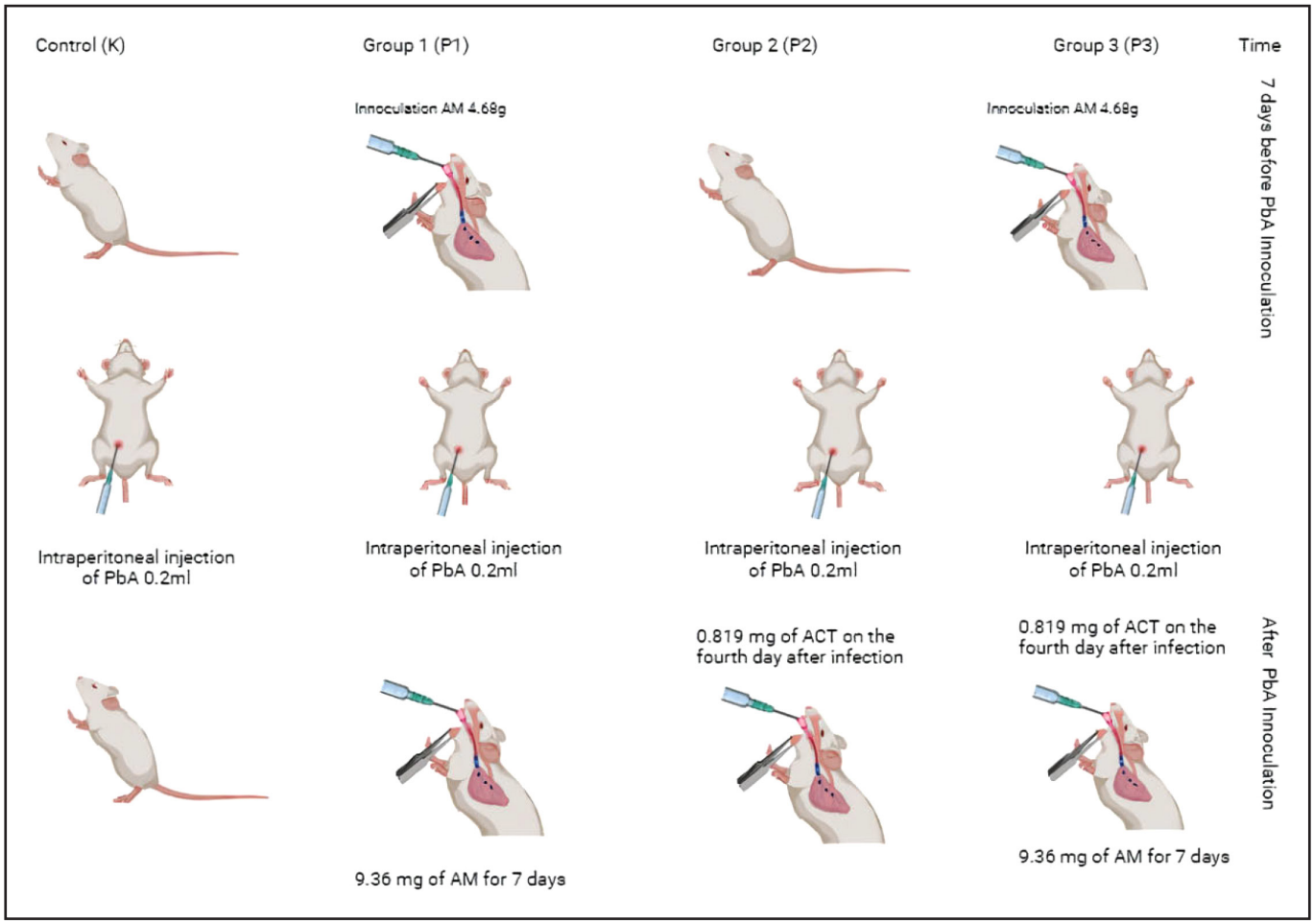

Figure 1. Treatment of mice.

Measurement CCL19 production capacity of spleen cells
To measure the CCL19 production capacity of spleen cells, the following steps were carried out:

1. Isolation of Cell Culture Supernatant:
   – Cultured cell supernatant stimulated by phytohaemagglutinin (PHA) and bacterial lipopolysaccharide (LPS) was isolated.
2. Storage:
   – The culture supernatant was properly stored at -80°C to maintain sample integrity.
3. CCL19 Measurement:
   – The levels of CCL19 were measured in the spleen cell culture supernatant using a mouse ELISA kit, specifically the Quantikine kit from R & D Systems.
4. Optical Density Reading:
   – The Optical Density of the samples was read using a microplate reader.

These steps allowed for the quantitative assessment of CCL19 levels in the spleen cell culture supernatant, providing valuable data for the research.

Data analysis
In the data analysis phase, the following statistical methods and tools were applied:

1. Bonferroni Post Hoc Test:
   – To investigate the difference in the mean of each group, the Bonferroni Post Hoc Test was employed. This test helps identify significant differences between group means
2. Kruskal Wallis Test:
   – Given that the distribution of the ratio scale data was not normal and the variance of the data remained unequal even after transformation, the Kruskal Wallis test was utilized. This test was used to assess whether there were differences in the medians among the four research groups.
3. Mann-Whitney Test:
   – To determine the size of the median within each group, the Mann-Whitney test was conducted. This test is particularly useful when comparing two independent groups.

All statistical analyses were carried out using the SPSS program. The significance level for this study was set at a p-value of less than 0.05, indicating statistical significance.

RESULTS

It's significant to note that all mice in each group successfully survived until the end of the study. Following the inoculation with PbA, all mice were confirmed to be infected, as evidenced by the examination of thin blood preparations taken from the tail on the 3rd day of PbA infection. Notably, groups K and P1 displayed notably high levels of parasitemia on the 7th day of PbA infection. In contrast, groups P2 and P3 exhibited very low parasitemia on the 7th day of infection. It's important to highlight that this study on CCL19 levels was conducted as a follow-up study and utilized spleen culture supernatants that were isolated from mice in prior research. This context provides valuable insights into the research process and outcomes.

Descriptive analysis of CCL19 levels of spleen products
A descriptive analysis of the CCL19 levels in spleen products was conducted, and the results were visually represented through a box plot in Figure 2. The box plot revealed that in the P3 group, the CCL19 levels were the highest compared to the other groups. This indicates that the group treated with a combination of AM (*Annona muricata*) and ACTS (*Artemisinin-Based Combination Therapy*) produced higher levels of CCL19 in comparison to the control group and the groups treated with AM or ACT alone. The box plot visually demonstrates the variations in CCL19 levels among the different treatment groups, highlighting the potential impact of the combination treatment on CCL19 production.

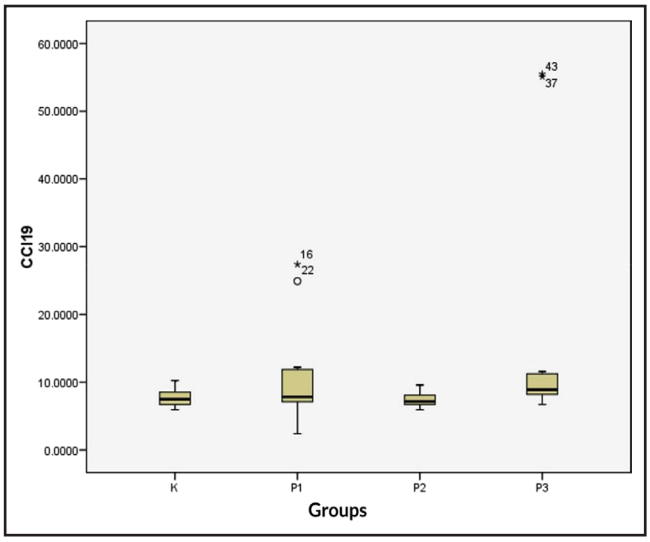

Figure 2. Box plot CCL19 levels of spleen products.

Table 2. Mann-Whitney Test results

Group	Medium (min-max)	P1	P2	P3
K	7.48 (5.96 – 10.26)	0.279	0.793	0.009*
P1	7.84 (2.40 – 27.37)		0.295	0.172
P2	7.15 (5.96 – 9.59)			0.002*
P3	8.90 (6.73 – 55.51)			

Note: * Significant ($p < 0.05$).

The analysis of CCL19 levels revealed interesting findings. The Shapiro-Wilk normality test indicated that only CCL19 levels in the P2 group followed a normal distribution (p=0.394), while the other groups displayed non-normally distributed CCL19 levels (K, p=0.018; P1, p=0.004; and P3, p<0.001). Given the abnormal distribution of CCL19 levels in the experimental animal groups, a non-parametric test, specifically the Kruskal-Wallis test, was employed to assess the hypothesis. The results of this test demonstrated a significant difference among the four research groups (p=0.015). Subsequently, further analysis between the two groups was conducted using the Mann-Whitney U test (Table 2). Notably, the CCL19 levels in the P3 group were higher and significantly different from those in the K group (p=0.009) and the P2 group (p=0.002).

These statistical findings provide valuable insights into the variation in CCL19 levels among the experimental groups, with a particular emphasis on the significantly higher levels observed in the P3 group in comparison to other groups.

DISCUSSION

The increase in the chemokine CCL19 is important to study in malaria for two reasons: CCL19 is a chemokine that mobilizes naive T-cells to come to secondary lymphoid organs, including the spleen (Masters *et al*., 2018). Another crucial reason is that the adoptive transfer of CD4+ Th cells prevents ECM and even increases PbA elimination in susceptible mice (Zotes *et al*., 2013). This AM-ACTs study showed that the CCL19 levels of the AM-ACTs combination treatment group

were higher and significantly different from the ACTs treatment alone (Table 2).

Mice received AM for 7 days prior to PbA inoculation, followed by 7 days of treatment post-inoculation, while the ACT was administered on day 4 of the PbA infection. This study successfully demonstrated that AM treatment increased spleen CCL19 levels in Swiss mice treated with ACT. The results indicated that there was no significant difference in CCL19 levels between the control group and the ACT treatment group. The measurement of spleen CCL19 levels was conducted on day 7 of PbA infection, during which the control group exhibited a high level of parasitemia, whereas the ACT group was in the healing phase with very low parasitemia levels. These findings suggest that increased splenic CCL19 levels were not observed in Swiss mice with severe PbA infection.

Spleen CCL19 levels in the AM-ACT combination treatment group were significantly higher than those in the control group ($p = 0.009$). This finding further supports the existing evidence that the AM-ACT combination treatment leads to an increase in spleen CCL19 levels. In contrast, the spleen CCL19 levels in the AM treatment group were higher than those in the control group, but the difference was not statistically significant ($p = 0.279$). This result underscores the notion that AM alone may not be sufficient to elevate spleen CCL19 levels in Swiss mice with PbA infection, highlighting the need for its combination with ACT.

Additionally, this study revealed that AM increased splenic CCL19 levels only during the healing phase of PbA infection when the level of parasitemia was very low. It's worth noting that the study employed AM treatment in combination with ACT administration on day 4 of PbA infection to maximize the protective effect of AM, which may have been more pronounced if the combination of ACT administration had been carried out on day 7 of PbA infection when the level of parasitemia was very high.

Evidence of the effectiveness of AM-ACT in increasing spleen CCL19 levels in mice with PbA infection complements previous studies using ethanol extract from the leaves of *A. Muricata* in Swiss mice with PbA infection. The study employed Swiss mice inoculated with PbA because they were considered susceptible to ECM after PbA inoculation (Utami *et al*., 2020). Ethanol extract from the leaves of A. Muricata has been shown to function as an immunomodulator, enhancing the immune system (Wahab *et al*., 2018). An increase in IL-10, IL-27, and CXCL12 during severe PbA infection was observed in Swiss mice treated with EDAM (Onohuean *et al*., 2021). This study used AM because AM has been widely circulated in the community so it is necessary to study its benefits, especially in cerebral malaria. Various studies show that there is no significant difference between water extract from the leaves of *A. Muricata (*AM) and ethanol extract from the leaves of *A. Muricata* (Vijayameena *et al*., 2013).

The chemokine CCL19 is expected to have various roles in assisting the immune response against cerebral malaria. This expectation is based on evidence that the number of specific naive T cells migrating to the spleen is very small (Zotes *et al*., 2013) and CCL19 accelerates the migration of unstimulated naive T cells and allows dendritic cells to produce proinflammatory cytokines and support Th1 cell formation (Wahid, 2016). The chemokine CCL19 has a dual role, promoting immune and immunosuppressive responses. CCL19 affects the activity and development of IL-10-producing Th2 cells (Wahid, 2016). IL-10 is an anti-inflammatory cytokine that has an important role in malaria cure (Kumar *et al*., 2019). IL-10 plays an important role in inhibiting the occurrence of ECM in the co-infected state of non-lethal malaria parasites, this may be due to its inhibitory effect on the induction of TNF-a and IFN- (Kumar *et al*., 2019). PbA infection in KO-CXCL10 mice suggests that increased expression of FoxP3, IL-10, and IL-2 by Treg cells may prevent the development of ECM in KO-CXCL10 mice(Kumar *et al*., 2019). Previous studies have also shown an increase in IL- 10 spleen production during the ECM phase by EDAM (Djamiatun *et al*., 2018). CCL19 levels affect the number and function of Treg cells (Campbell, 2015).

IL-10 production is stimulated by IL-27 as a major regulator of production by human CD4+ T cells (Sukhbaatar *et al*., 2020). Other studies have also shown that IL-27 induces the production of IL-10 by T lymphocytes and reduces Th1, Th2, and Th17 responses. IL-27 also promotes IL-10 production by various populations of effector CD4+ T cells, including Th1 and Th2 cells (Findlay *et al*., 2019). IL-10 can be produced by Tr1 cells and allows IL-10 to suppress the response of T-cytotoxic (Tc) cells. Tr1 cells can be derived from naive CD4+ T cells after IL-27 stimulation or through the role of CD55 as a co-stimulator (Sutavani *et al*., 2013). This indicates the role of CCL19 and IL-27 against IL-10 which is a cytokine that plays an important role in the immune response to cerebral malaria. The results of previous studies showed that EDAM increased IL-27 during ECM (Djamiatun *et al*., 2018). There is no evidence that AM-ACT increases IL-27 and IL-10 in the spleen of mice with PbA infection.

Anonna muricata leaf water extract contains more than 200 chemical compounds that have been identified and isolated from plants, the important compounds of which are alkaloids, phenols, acetogenins, flavonoids, and terpenoids. Several studies have shown that chemical compounds in the form of flavonoid alkaloids, terpenoids, polysaccharides, lactones, and glycoside products are responsible for causing changes in immunomodulatory properties (Sharma *et al*., 2017). The study also demonstrated that AM enhances immunity through activation of the mitogen-activated protein (MAP) kinase pathway. The bioactive properties of AM are to stimulate immune potential and enhance the innate immune system even in immunocompromised patients (Gavamukulya *et al*., 2017).

The protective effect of AM on survival and parasitemia levels would be more obvious if the combination of AM and ACT was administered on the 7th day of PbA infection where the parasitemia level was very high, but in this study, AM combined with ACT was performed on day 4 of PbA infection. This is a limitation of this study. Another limitation is that the study only measured the effectiveness of AM-ACT on CCL19 levels. The effectiveness of the AM-ACT combination on various protective biomarkers (CXCL12, IL-27, and IL-10) in Swiss mice with PbA infection would strengthen the evidence for the protective effect of the combination.

This study also only counted the increase in CCL19 levels, while the number of naive CD4+ Th cells or Th1 cells was not counted. The correlation of the number of CCL19 levels, naive CD4+ Th cells, or TH1 cells will complete this study because one of the functions of CCL19 is to accelerate the migration of naive T cells that have not been stimulated and allow dendritic cells to produce proinflammatory cytokines and support the formation of Th1 cells. Previous studies have shown an increase in the expression of FoxP3, IL-10, and IL-2 by Treg cells which may prevent the development of ECM in PbA infection of KO-CXCL10 mice, so the evidence that will complete the study is the correlation between CCL19 and FoxP3 levels in the AM-ACT combination treatment. CCL19 is produced in splenic dendritic cells, in this study CCL19 was calculated from the spleen culture supernatant but it was known that there were many other cells in the spleen, so CCL19 levels would be clearer if calculated by immunohistochemical methods.

Furthermore, the utilization of *Annona muricata* leaf water extract offers advantages in terms of accessibility, particularly in malaria-endemic areas like sub-Saharan Africa and Papua (Indonesia). Herbal medicines are frequently the first line of treatment due to their affordability, availability, perceived effectiveness, minimal side effects, and the trust placed in traditional remedies by local communities (Tajbakhsh *et al*., 2021). Dunst *et al*. (2017) explained the need for adjunctive therapies to prevent adverse effects of the immune response to *Plasmodium* infection, particularly in severe cases such as cerebral malaria. They suggested that immunomodulation is a promising approach to alleviate immune-mediated pathology, but such therapies need to be designed carefully to maintain efficient control of parasite growth.

The document also mentions that adjunct therapies modulating chemokine responses may have fewer side-effects compared to therapies based on neutralizing cytokines. However, the document does not provide specific recommendations for adjunctive therapies for malaria (Dunst *et al*., 2017).

CONCLUSION

Based on this study, it can be concluded that the CCL19 levels of Swiss mice with PbA infection in the effective treatment group increased with the AM-ACT combination. Although it still requires further research, this study shows that the water extract of *Annona muricata* leaves increases chemokines which have the function of helping naive T cell migration to improve the immune system. While *Annona muricata* leaf water extract shows promise as a potential source of new antimalarial drugs, more research is needed to understand its mechanisms of action and potential side effects. Additionally, any new antimalarial drugs derived from *Annona muricata* leaf water extract would need to be rigorously tested in clinical trials to ensure their safety and efficacy.

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Conflict of Interest

All authors declared that there are no competing interests.

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