ORIGINAL ARTICLE:

Sambiloto (AS201-01) is better than standard antimalarial drug (DHP) in reducing Toll-Like Receptor 2 (TLR2) on placenta malaria model

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ABSTRACT

Objectives: To compare the TLR2 expression in the placenta between treated by *sambiloto* EA-96 fraction tablet (AS201-01) and dihydroartemisinin-piperaquine phosphate (DHP)
Materials and Methods: Experimental study using 24 pregnant mice. All sample divided into 4 groups with randomization are uninfected group, Plasmodium infected group and given placebo, *sambiloto* (AS201-01) and DHP. Then performed surgery and placental sampling were staining with adopting in tunnel assay method to measure the TLR2 expression of placental.
Results: The expression of TLR2 in uninfected group has the lowest rate compared to other groups. The infected and placebo treated group has the highest TLR2 expression campared with *sambiloto* and DHP. The *sambiloto* group has not differ significantly with the group uninfected and lower than DHP.
Conclusion: Tablet of *sambiloto* EA-96 fraction (AS201-01) decreased TLR2 expession better than with DHP tablet.

Keywords: Placental malaria, TLR2, *sambiloto* (AS201-01), DHP

ABSTRAK

Tujuan: Membandingkan ekspresi TLR2 pada plasenta mencit bunting diinfeksikan *P. berghei* yang diberi tablet fraksi EA-96 *sambiloto* (AS201-01) dengan yang diberi *dihydroartemisinin-piperaquine phosphate* (DHP)
Bahan dan Metode: Eksperimental laboratorium, 24 ekor mencit bunting dibagi dalam 4 kelompok dengan randomisasi yaitu kelompok tidak diinfeksikan *Plasmodium*, kelompok diinfeksikan *Plasmodium* dan diberi plasebo, *sambiloto* (AS201-01) dan DHP. Kemudian dilakukan pembedahan, diambil sampel plasenta, dilakukan pewarnaan imunohistokimia dan dihitung ekspresi TLR2.
Hasil: Ekspresi TLR2 pada kelompok tidak diinfeksikan *Plasmodium* merupakan rerata terendah dibandingkan dengan kelompok lainnya. Pada kelompok yang diiinfeksikan *Plasmodium* dan diberi plasebo memiliki ekspresi TLR2 tertinggi dibandingkan *sambiloto* dan DHP. Kelompok *sambiloto* tidak berbeda bermakna dengan kelompok yang tidak diiinfeksikan *Plasmodium* dan lebih rendah dibandingkan dengan DHP.
Simpulan: Tablet fraksi EA-96 *sambiloto* (AS201-01) menurunkan ekspresi TLR2 lebih baik dibandingkan dengan tablet DHP

Kata kunci: Malaria plasenta, TLR2, *sambiloto* (AS201-01), DHP

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INTRODUCTION

Malaria is an important public health problem and a major concern world health. Women are more susceptible to malaria during pregnancy and in the puerpurium[1]. At least 50 million pregnancies are exposed every year to malaria infection, can even cause death up to 50% higher than non-pregnant women and fetal death with an estimated mortality of 200.000 per year. Pregnancy malaria relates to malaria attributable maternal anaemia, which may be severe and increase maternal morbidity and the risk of mortality. The consequences of pregnancy malaria and placental malaria for the fetus and infant are enormous.[2-6] Anemia, PTD (preterm delivery), and FGR (fetal growth restriction) are major causes of malaria-associated low birth weight (LBW) babies[8]. These infections may result from single or mixed infections with any of the four species of *Plasmodium* which cause human malaria. These are *P. falciparum, P. vivax, P. ovale* and *P. malariae.*[1] *Plasmodium falciparum* is known to cause the most severe form of malaria.[8]

The availability of novel placental receptors may select for malaria parasites that are uncommon in non-pregnant hosts and this could increase the density and duration of placental malaria. These consequences indicate that placental malaria presents a unique set of problems.[1]

Placental malaria changes the environment in the intervillous space of placenta. It occurs as a result of *P. falciparum* infected erythrocytes (IE) binding to syncytiotrophoblast (ST), a continuous, multinucleated, specialized epithelia layer that covers interior of the villous of the placenta. *P. falciparum*-IEs specifically bind on ST receptors known as chondroitin sulphate A (CSA) and hyaluronic acid. Binding of IE to ST leads to sequestration of IE in the intervillous space (IVS) of the placenta. The ability of IE to bind on CSA is conferred by the parasite's variant surface antigen belonging to var. gene subfamily (VAR2) that is expressed on the surface of IE called VAR2CSA. Generally, sequestration of IE in the IVS leads to secretion of chemokines resulting in inflammatory cells recruitment and cytokine production which is associatedwith poor pregnancy outcomes.[8]

Syncytiotrophoblast in placenta serves as a barrier that separates the maternal and fetal blood so as to prevent the transmission of pathogen from mother to fetus, including erythrocytes infected with *P. falciparum*. Among them, generating an immune barrier against pathogens, such as the innate immune system are pattern recognition receptors (PRRS), including Toll-like receptors (TLR1-10).[8] TLR2 is activated by the key malaria toxin that is *Glycosylphosphatidylinositol* (GPI)[9].

TLR activation is mediated by a variety of adapter proteins, such as Myeloid Differentiation Factor 88 (MyD88), Tirap (Mal), Trif, and Tram and culminating in a proinflammatory gene expression, such as TNF-α, interferon-gamma (IFN-γ) and IL-12. The expression of these cytokines is accompanied by accumulation of parasites can cause placental hypoxia. In addition, cytokines can also affect nutrient transport mechanisms. All these changes that ultimately can affect the growth janin in the form of stunted growth of the fetus in utero (IUGR), low birth weight (LBW), and miscarriage. However, the contribution of TLR2 in the pathogenesis of malaria still kontroversial.[9-11]

Malaria morbidity and mortality can be reduced by means of appropriate and rapid treatment. In the past, malaria treated with chloroquine. After reports of resistance, treatment with the combination developed namely ACT (Artemisinin-based Combination Therapy). Starting in 2011 Dihidroartemisinin-piperaquine (DHP) used nationwide.[33] The use of artemisinin in pregnancy remains controversial. On the one hand there is evidence of significant teratogenicity in animal models. On the other hand a thousand pregnant women have followed treatment with artemisinin reported that they had no problem with her pregnancy.This is a serious challenge to the effectiveness of the malaria program in pregnancy.[5,6]

Sambiloto is a natural ingredient that has been used by the community as a malaria treatment. Antimalarial drugs natural ingredients are more stable and have lower side effects than synthetic materials. The use of *sambiloto* as antimalarial being developed mainly in pregnancy.[12,13] Research in Surabaya have previously found that an extract of *sambiloto* can inhibit the growth of *P. falciparum* in vivo and have the same effectiveness as chloroquine diphosphate. At security test are sub acute toxicity tests on tablets diterpene lactone fraction obtained AST, ALT, ureum, creatinine and liver histopathology normal picture. Extract of *sambiloto* in the form of fraction of ethyl acetate (EA) 96% shown to inhibit growth *P. berghei* in mice up to 94.36%.[14]

Potential tablet EA fraction 96% *sambiloto* as malaria treatment in pregnancy with an equivalent dose of 25mg andrografolida/ kg given twice daily in pregnant mice can inhibit parasite growth by the barriers at 17.54% and provide protective effects on the fetus, shown by the absence of change morphology of fetal mice infected with *P. berghei* is long skull bones, scapulae, costae, vertebrae, humerus, radius ulna, femur and tibia-fibula.[15] The tablet also can reduce the accumulation of

placental malaria parasites in pregnant mice were infected *P. berghei*.[16] As with placental apoptosis index in the delivery of 96% EA tablet *sambiloto* fraction lower than those given DHP[17]. Studies using *P. berghei* infection because the infection resemble *P. falciparum* on humans.[4]

MATERIALS AND METHODS

This study design is a laboratory experimental study on mice (*Mus musculus*) strain BALB/c are trying randomized with control samples for comparison. This research was conducted at the Institute of Tropical Disease (ITD), Universitas Airlangga, Laboratory of Veterinary Pathology, Faculty of Veterinary Medicine, Universitas Airlangga. The study population was mice (*Mus musculus*) strain BALB/ c units animal laboratory where the mice have similarities with humans.

The research sample weight of 24 pregnant mice are 25-35g and meet the inclusion and exclusion criteria were divided into 4 groups of 6 animals each, ie K1, K2, K3, K4. K1 groups are: pregnant mice without infection *P. berghei*, K2: pregnant mice with infection who received placebo Carboxymethyle *P. berghei* Cellulose Sodium (CMC Na) 2x a day for 4 days (placebo group). K3: pregnant mice with an *P. berghei* infection that gets tablet *sambiloto* (AS201-01) andrografolida dose of 25 mg/ kg 2x a day for 4 days (group AS201-01). K4: pregnant mice with *P. berghei* infection that gets tablets dihydroartemisinin - piperaquine phosphate 1x daily for 3 days (Group DHP). K2, K3, K4 on day 11 paracytaemia examination, if positive given the treatment, the 15th day of surgery, a swab of the placenta, made preparations blocks of paraffin and inspection performed IHC staining for TLR2.

Tablet *sambiloto* (AS201-01) tablet is 96% ethyl acetate fraction obtained from extraction of *sambiloto* leaf that is done, fractionation with ethyl acetate and then formulated in tablet form. Used andrografolida dose of 25 mg/ kg. DHP used dose tablet 1.248 m/ kg/ day.

TLR2 expression calculation using the Immuno Reactive Score is the multiplication of cells immunoreactive with a percentage score of the immunoreactive stain intensity cells in IHC staining. Placental tissue consisting of cells experiencing DNA fragmentation syncytiotrophoblast will give color brown chromogen.

This research was recorded in a data collection form. Data analysis used statistics application software package. Test for normality was done using the Kolmogorov-Smirnov test. When the normal distribution of data followed by Anova. The significance level used in this study amoun-ted to 0.05. Conduct a feasibility study is obtained from the ethics committee of the Faculty of Veterinary Medicine Airlangga University (No. 725-KE).

RESULTS AND DISCUSSION

Characteristics of the parent mice prior to treatment based on weight as in the Table 1.

Table 1. Weight loss pregnant mice strain BALB/c were infected with *P. berghei*

Mothers' Body Weight (g)	K1	K2	K3	K4	P value
Mean	30.83	30.50	31.83	31.17	0.89
SD	1.72	3.08	4.26	2,48	

Data mice body weight before treatment normality test with Kolmogorov-Smirnov test. From a statistical calculation showed that no significant difference (P>0.05) in the groups of mice before treatment, indicating homogeneous samples.

Analysis of expression of TLR2 in placenta of pregnant mice

In this study, 24 pregnant mice who performed surgery on day 15, and then taken to be made preparations placenta tissue paraffin block, then do Hematoxilin - eosin staining and immunohistochemistry followed by antibody staining for TLR2. Then examined under a microscope to see the expression of TLR2. Placental tissue examination conducted in the laboratory of Veterinary Pathology, Faculty of Veterinary Medicine, Universitas Airlangga.

Figure 1 shows placental cells that express TLR2. From the above description it appears that the amount of expression of TLR2 (chromogen color brown) in the placebo group more than the DHP. TLR2 expression in tablet DHP more than *sambiloto* (AS201-01), and the expression of TLR2 *sambiloto* (AS201-01) more than those uninfected group

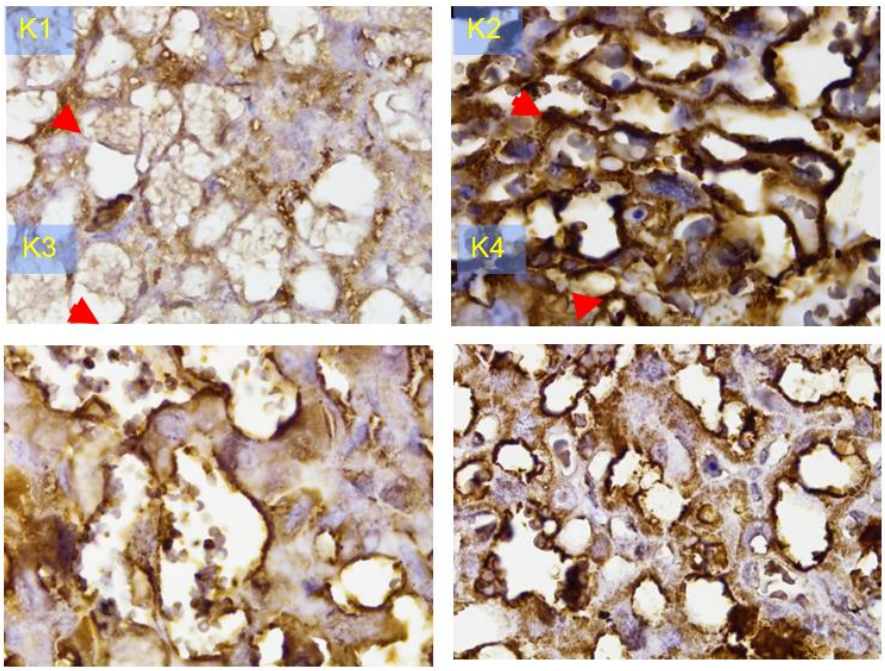

Figure 1. Toll Like Receptor2 (TLR2) IHC photo placental cells pregnant mice

Notes: Differences in the expression of TLR2 on syncytiotrophoblast cells (arrows) between the treatment groups. In the slide above it appears that the most powerful expression of TLR2 occur in K2 compared to the other treatment groups (immunohistochemical staining, magnification 1000x; H600L Nikon microscope; Fi2 300 megapixel camera DS); K1: group uninfected by Plasmodium; K2: placebo; K3: *sambiloto* (AS201-01); K4: DHP tablet group.

TLR2 expression data on placental cells performed the Kolmogorov-Smirnov normality test, and showed that the normal distribution of data. Statistical analysis for the different test Anova. TLR2 expression on placental cells was calculated by summing the cells undergo DNA fragmentation, cells expressing TLR2 calculated at 5 field of view with a magnification of 1000x. Results of TLR2 expression in placental cells in each group as shown in Table 2.

In the above statistical data it appears that the average expression of TLR2 in the uninfected group Plasmodium is the lowest average and is the highest in the placebo group. The mean expression of TLR2 in the uninfected group was not significantly different Plasmodium with *sambiloto* tablet group (AS201-01) and significantly lower than the placebo group and DHP. *Sambiloto* tablet group (AS201-01) has TLR2 expression results were not significantly different with Plasmodium uninfected group (P>0.05) but lower when compared with the placebo group and DHP (P<0.05). When compared between the placebo group and the DHP tablets obtained TLR2 expression was lower in DHP tablet group compared with the placebo group (P, 0.05). Meanwhile, when compared between groups and DHP bitter tablets obtained TLR2 expression of bitter group is lower than DHP tablet group (P<0.05).

Table 2. Mean and standard deviation TLR2 expression on placental cells pregnant mice in each treatment group

Group	TLR2 Mean ± SD	p value
K1	5.87±1.63a	0. 00
K2	11.27±0.77b	
K3	6.17±1.60a	
K4	8.70±2.07c	

This research uses experimental 24 animals female white mice BALB/ c, with about 3 months of age, were selected as test animals because of similarities in terms of taxonomy and physiology of humans with mice gestation period of about 17-21 days. *Plasmodium berghei* infection in pregnant mice, which are character-istic of the accumulation of erythrocytes were also obtained as in *P. falciparum* that infect humans.[4]

In this study, 24 mice strain BALB/ c that meet the inclusion criteria, selection of mice that either uniform weight and age. Pregnant mice were randomly divided into 4 groups by weight mice used in this study approximately 25-35 grams. Statistical analysis found no significant differences in the groups of mice prior to treatment, within the meaning of homogeneous groups (P>0.05).

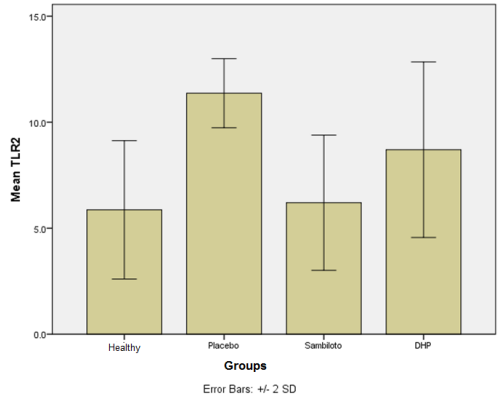

Figure 2. Diagram the average expression TRL2 in each group

Figure 2 shows that are TRL2 expression in the group has *sambiloto* tablet (AS 201-01) similar to uninfected Plasmodium. While those with the highest TRL2 expres-sion in placebo group.

This study used mice experiencing first pregnancy, because the difference of parity relates to the ability of local produce cytokines present in the placenta. In the multigravida able to produce cytokines IFN-γ higher than primigravida, so as to eliminate malaria infection, whereas in mice primigravidas issued cytokine IFN-γ slower and less. Cytokine IFN-γ will stimulate monocytes to secrete proinflammatory cytokines. Cytokines that will eliminate the malaria parasite by increasing the activity of macrophage and T cell stimulation. In addition, serum from malaria-infected pregnant women have the ability to inhibit the attachment of the parasite in Chondroitin sulphate-A (CSA). The level of inhibition is found to be higher in comparison with primigravida than the multigravida.[18]

Effect of Toll Like Receptor2 (TLR2) against infection of *P. berghei*

Erythrocytes infected the parasite will produce a toxin called as glycosyl phospatidylinositol (GPI) where the toxin binds to the receptor, namely CD14 which then activate macrophages and immune system, others are (TNF-α, IL-1, IL-6, IL-8, IL -12, IFN-γ). Likewise, the surface of the parasite-infected erythrocytes to be raised bumps called the knob, where on the knob there are a variety of proteins including the *P. falciparum* erythrocyte membrane protein-1 (PfEMP-1) which is the most important protein in sitoadherens produced by erythrocytes infected with the malaria parasite. Antigen protein would act as a parasite ligands by receptors identifiers such as Toll-like receptors (TLRs), CD36, thrombospondin, Chondroitin Sulphote-A (CSA), hyaluronic acid, P-selectin and CRI, which mediates rosetting and the establishment of agglutination or inflammatory cytokines such as IFN-Y, dendritic cells and migrate to the spleen which is a major cycle of immune response to the erythrocytic phase of Plasmodium parasite[19].

Previous research conducted on mice showed that the *P. falciparum* GPI specifically recognized by TLR2 and TLR4 is a signal that induces the production of proinflammatory cytokines.[20,21] Hypothesis in a study conducted at the outbreak of the schizonts obtained, releasing hemozoin and/or GPI induces differences TLR expres-sion which then induces the production of cytokines pro inflamatory[9].

Namely TLR innate immunity, especially TLR2 recognizes GPI (typical toxin *P. falciparum*), produced by trophoblasts. Syncytiotrophoblast is a barrier, which separates the maternal and fetal blood. Normally syncytiotrophoblast prevent bloodborne transmission of an infection from mother to fetus including erythrocytes infected with P. placenta falciparum.[8]

Many studies indicate that these products are not malaria can accumulate in fetal structures. Although malaria parasites are detected, in small amounts, ie cord blood viewed under a microscope with Giemsa, malaria parasites are never described in fetal erythrocytes or fetal structures in histology studies. It is stressed that the malaria parasite can not pass the placental barrier to the delivery, which is when a ruptured blood vessel and mixed between fetal and maternal blood. Based on previous studies, mode of delivery (MOD) effect on placental histopathology invention including their picture sekuestrasi[1].

In Table 2 it can be seen that the average expression of TLR2 on placental cells K1 group (group uninfected by Plasmodium) is the average of the lowest when compared with the group of pregnant mice were infected with *P. berghei*, while the placebo group was the highest. Significant differences between the group results were not infected with *P. berghei* infected means that the group try K2, K3 and K4 is a group that has been infected by Plasmodium and the continuation of treatment in each group. Sequentially from the K2-K4 is given a placebo, *sambiloto* (AS201-01) and DHP.

In Figure 1 looks picture placental cells with chromogen color brown, which illustrates the fragmentation of DNA labeled with toll-like receptor2 (TLR2). This proves that the pregnant mice without infection or with malaria infection TLR2 expression on placental cells. TLR2 expression on placental cells pregnant mice given malaria infection also proved to be higher compared with the uninfected group Plasmodium malaria.

Previous research found TLR4 expression in pregnant mice with the average of the entire sample group is 2,50.22 mean in the whole group over the study found lower than TLR2 expression in this study (8.00). This is in accordance with Loharungsikul 2008, Glycosyl-phosphatidylinositol (GPI) is a glycolipid of a surface protein toxin merosoit (merozoite surface protein/MSP-1 and MSP-2), recognized mainly by TLR2 and little by TLR4.[9]

In the study Barboza R., et.al. shows that the development of placental malaria occurs because TLR induces excessive local inflammation. These findings indicate a significant relationship to malaria in pregnancy and the release of cytokines by the placenta where TLRs act as a sensor of placental tissue from Plasmodium, through MyD88 pathway, triggers the release of inflammatory cytokines in the maternal blood. Inflammation that occurs as a result of the activation of the innate immune system through TLRs have a negative effect on the results kehamilan[10].

Christian (2005) and Michael (2010) said that TLR2 on the surface of the membrane, binds to Plasmodium-2-cys peroxidoxinen and recognize GPI, antigens from parasites, through myeloid differentiation factor 88 (MyD88). Which is then accompanied by lines of MAPK and nuclear factor-kappa B (NF-kB) activation stimulates proinflammatory factors such as cytokines, TNF-α and IFN-γ.[23-25]

Found in elevated levels of TNF-α in systemic maternal, placental malaria from infected mice showed increased expression of TNF-α mRNA. What is interesting is both the systemic and local, increased expression of pro-inflammatory cytokines dependent on MyD88, which shows the state in which the adapter protein is not found, this also had a negative effect on the immune response, it is found in studies by Franklin et.al.[10]

The effects on the infants in the study Barboza R., et.al. using mice infected with *P. berghei* obtained infant birth weight lower than control and is associated with the expression of MyD88, wherein the protein that is not found this to happen a significant reduction in inflammatory factors (IL-6 and TNF-α) in the placenta and baby weight was affected.[10]

Research on placental malaria pathogenesis is required for the development of therapeutic strategies, in order to get the main line of stimulation by parasites that move the innate immune system is important in the local inflammatory response. Consequently, by deciding the track could be critical approach in the treatment of placental inflammation induced by Plasmodium in the treatment of malaria in pregnancy[10].

Comparison of TLR2 expression in the placenta between the group that received a placebo, tablet fraction of EA-96 (AS201-01) *sambiloto* and dihydroartemisinin-piperaquine phosphate (DHP)

In the results, the mean expression of TLR2 in the placebo group (K2) is 11.27±0.77, the mean expres-sion of TLR2 in the *sambiloto* tablet group (AS201-01) (K3) is 6.17±1.60 and TLR2 expression in DHP tablet group (K4) was 8.70±2.07. Test normality with the Kolmogorov-Smirnov test result is normally distributed data $p>0.05$. Results of different test Anova p value< 0.000 showed significant differences among the three groups.

TLR2 expression in the placenta in tablet *sambiloto* (AS201-01) (K3) lower than the placebo group (K2), the results obtained by different test p value of 0.000 ($p<0.05$). This shows that there is a significant difference between the placebo group and the group of *sambiloto* tablet (AS201-01). Likewise the *sambiloto* tablet group (AS201-01) (K3) has a TLR2 expression lower than the group of tablets DHP (K4) with the results of different test p 0.012 ($p<0.05$), so it can be concluded significant differences between K3 and K4. While the tablet group DHP (K4) compared with the placebo group (K2) showed different test p 0.011 ($p<0.05$) so that we can conclude there are also significant differences between the two groups.

Giving tablet fraction of ethyl acetate-96 (AS201-01) *sambiloto* on this study, a decrease in the expression of TLR2 in the mice placenta infection compared with placebo treatment group and dihydroartemisinin piperaquine phosphate group. It can be concluded that the provision of standardized *sambiloto* tablet can affect a decrease in TLR2 expression in the placenta of mice infected with Plasmodium.

Infected placenta associated with preterm labor. In a study obtained 81.1% placental infection occurs preterm labor. It supports an association between placental infection with preterm labor. TLR2 expression was also found in amniotic fluid and decidua cells increased in the state of infection in preterm labor. Thus concluded that TLR2 expression was higher in placental infection

which reinforced the role of TLR2 in preterm pregnancy the placenta and protect against infection.[26]

Prevention of malaria in pregnant women in Africa recommends using sulfadoxine-pyrimethamine. The study was conducted comparing the DHP with sulfadoxine-pyrimethamine. Histopathology of placental malaria, it was noted lower on DHP administration were compared with sulfadoxine-pyrimethamine. Whereas for fetal outcomes between the two treatment did not significant different.[27] Use of four ACT (artemether-lumefrantrine, amodiaquine-artesunate, DHP, mefloquine-artesunate) in pregnant women in Africa obtain DHP has the best efficacy figures (for treatment failure and clinical response and parasitology). There were no significant differences in outcomes between the babies in that four therapies.[28]

Dihydroartemisinin Piperaquine Phosphate (DHP) have inhibitory activity through inhibition of digestive tract parasites heme in the food vacuole. Andrografolida, is the main *sambiloto* compound are diterpene lactone compound with high levels of around 2.5% and is gametosida. The ethanol extract of *sambiloto* is known to inhibit the growth of *P. falciparum* parasites in vitro, while the main compound is andrographolide can inhibit 50% growth of *P. berghei* in vivo. From the results of these studies have also been able to isolate separated diterpene lactone that also have anti-malarial activity. Isolates diterpene lactone is able to inhibit the growth of *P. falciparum* gametocytes-stage in vitro, where this activity is equivalent to standard drug activity primaquine. These isolates also have schizontocide activity.[9]

This study found that TLR2 expression was lower in the group given fraction tablet therapy EA-96 (AS201-01) *sambiloto* with placebo and DHP. This is caused by a *sambiloto* has gametosida effect of androgapholida and act as an immunomodulator. The inhibitory effect of Plasmodium parasites in the blood by DHP is 100% whereas *sambiloto* is said to inhibit by 16%. However *sambiloto* may stimulate the immune system and activate the anti-inflammatory factors through *MyD88 and NF-κB*.[30] So that the TLR2 expression of placental tissue that indicate the existence of a bond with the parasite protein, GPI, fewer the *sambiloto* is fewest, compared with the DHP and placebo.

In a previous study using a sample of pregnant mice infected with *P. berghei* similar to our study, with an equivalent dose of 25 mg andrografolida/ kg can inhibit parasite growth by the barriers at 17.54% and provide protective effects on the fetus. The resulting barriers EA-96 tablet fraction *sambiloto* (AS201-01) is smaller than the resistance of the tablet ACT in the study. Studied the effect of tablet fraction of EA-96 (AS201-01) on fetal bone size and morphology of fetal mice, showed that there was no difference in the length of skull bones, scapulae, costae, vertebrae, humerus, radius-ulna, femur and tibia- fibula fetus pregnant mice infected with *Plasmodium berghei* between who got *sambiloto* with ethyl acetate fraction and uninfected Plasmodium and concluded there was no difference in fetal morphology pregnant mice infected with *Plasmodium berghei* between who got the ethyl acetate fractions *sambiloto* (AS201-01) and uninfected Plasmodium.[15]

Sambiloto is an antimalarial that can lower both TLR2 through the barriers in the process of degradation of hemoglobin and heme detoxification, where the heme itself triggers the activation of inflammatory cytokines. This is consistent with results of previous studies that Andrographolide have activity as schizontocide, working also on the parasite food vacuoles through inhibition of the polymerase heme.[29]

Wang et al reported that the *sambiloto* can maintain endothelial function and maintaining the balance of nitric oxide/endothelin. In addition *sambiloto*, wherein the extract of *sambiloto* derived from stems and leaves of 25 mg/ kg in mice could stimulate antibodies to mice.[30] It was also reported that the content of the andrografolide in *sambiloto* able to inhibit the production of TNF-α and IL-12 in macrophages.[31]

Their expression of TLR2 significant difference between groups tablets *sambiloto* (AS201-01) and DHP tablet indicates that the tablet *sambiloto* (AS201-01) able to reduce the expression of TLR2 in placental malaria better than the tablet DHP. Tablet DHP is a standard antimalaria combination drug, which has been much research done, proven to reduce blood paracitemia very significant compared to the other drug combinations. DHP is a combination tablet consisting of 40 mg and 320 mg dihydroartemisisnin piperaquine phosphate in the form of a single dose. This drug is an active metabolite of artemisinin works quickly eliminate parasites in the body, while piperaquine has a long half-life for 23 days (19-28 days). Artemisinin and piper-aquine have the same schizontocide effect, inhibiting the input of nutrients into the parasite food vacuole and also inhibits hemoglobinase (anti-malaria).

CONCLUSION

Expression of Toll-like receptor2 (TLR2) in the placenta of pregnant mice were infected by *P. berghei* and tablet therapy EA fraction 96 (AS201-01) *sambiloto* lower than that given therapy dihydroartemisinin-piper-

aquine phosphate (DHP). By decreasing the stimulation of TLR2 lowering proinflammatory cyto-kines that do not cause adverse pregnancy outcomes in placental malaria (miscarriage, low birth weight and IUGR)

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