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CONTENTS

Tumor Necrosis Factor-Alpha (TNF-Alpha) and Intercellular Adhesion Molecule-1 (ICAM-1) Expression of *Plasmodium berghei* Infected Swiss Mice Treated with Red Fruit (*Pandanus Conoideus* Lam) Ethanol Extract

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ABSTRACT

Introduction: Malaria infection could activate T cell helper 2 CD4+ which release cytokines IL-4, IL-5, IL-10 was a kind of immunosuppressant cytokines and prevented a cerebral tissue damage from the expression intercellular adhesion molecule-1 (ICAM-1) by TNF-α, ended with a cerebral malaria. The red fruit was proved as antioxidant but its effect to reduce TNF-α and expression of ICAM-1 in cerebral tissue endothelial cell remained unknown.

Objectives: to know the effect of red fruit ethanol extract toward the change of TNF-α secretion number and ICAM-1 in Swiss male mice's cerebral endothelial cell infected with *P.berghei*.

Methods: Sixty Swiss male mice were divided in to 6 groups, group I was not given the red fruit (BM) ethanol extract and not infected *P.berghei*, Group II was given BM 260mg/kg BB but not infected *P. berghei*, group III, IV, V were given BM 130, 260, and 520 mg/kg respectively and infected with *P. berghei*. Red fruit extract or carrier substance was given for 4 weeks, *P. berghei* was given in the early of third week. TNF-α level check was done in third and ninth day after the injection, with *TNF-α ELISA kit mouse*. ICAM-1 cerebral endotel cell expression check was done in third and nine day with immunohistochemistry (IHC) coloring.

Results: TNF-α level in third day after infected to the groups given red fruit extract (group III, IV, and V) showed no significant difference (p=0,839) compared to group which was not given red fruit but infected (group VI). TNF-α level in ninth day after infected to groups given red fruit (group III,IV,V) lower (p<0,05) compared to groups which was not given red fruit but infected. ICAM-1 expression in third and ninth day after treatment to group given red fruit extract was lower (p<0,05) compared to groups given red fruit extract but infected.

Conclusion: Red fruit ethanol extract (*P conoideus* Lam) could decrease TNF-α level and ICAM-I expression in Swiss mice' endothelial cerebral cells after infected by *P. berghei*.

Key word: *Pandanus conoideus* Lam, TNF-α, ICAM-1 expression, *Plasmodium berghei*, Swiss mice

INTISARI

Pendahuluan: Infeksi malaria dapat mengaktifkan sel T helper 2 $CD4^+$ yang berfungsi melepaskan sitokin IL-4, IL-5, IL-10 merupakan sitokin imunosupresan dan mencegah kerusakan jaringan otak akibat terekspresinya *interceluler adhesion molecule-1* (ICAM-1) oleh TNF-α, sehingga menyebabkan serebral malaria. Pemberian ekstrak buah merah pada infeksi malaria *Plasmodium* dapat meningkatkan proliferasi sel limfosit T helper 2 $CD4^+$. Hal ini karena buah mengandung senyawa aktif betakaroten, tokoferol, asam askorbat yang tinggi yang terbukti memiliki kemampuan untuk berinteraksi sebagai anti inflamasi dengan meregulasi protein M2 yang mengaktifasi NFAT untuk meregulasi IL-10 yang menghambat produksi TNF-α. Buah merah juga terbukti memunyai aktivitas sebagai antioksidan. Namun, efek buah merah dalam mengurangi TNF-α dan ekspresi ICAM-1 di sel endotel jaringan otak belum diketahui.

Tujuan: Untuk mengetahui efek ekstrak etanol buah merah terhadap perubahan jumlah sekresi TNF-α dan ekspresi ICAM-1 di sel endotel jaringan otak mencit Swiss jantan yang diinfeksi *P. berghei*.

Metode: Penelitian ini menggunakan rancangan eksperimen kuasi dengan *post test only control group design*. Enam puluh ekor mencit Swiss jantan dibagi menjadi 6 kelompok, kelompok I tidak diberi ekstrak etanol buah merah (BM) dan tidak diinfeksi *P.berghei*, kelompok II diberi BM 260 mg/kg BB, namun tidak diinfeksi *P. berghei*, kelompok III, IV, V diberi BM berturut turut 130, 260, dan 520 mg/kg BB selanjutnya diinfeksi *P. berghei*. Kelompok VI tanpa BM namun diinfeksi *P. berghei*. Ekstrak buah merah diberikan selama 4 minggu, infeksi *P. berghei* dilakukan pada awal minggu ke-3. Pemeriksaan kadar TNF-α dilakukan pada hari ke-3 dan hari ke-9 setelah infeksi, dengan *mouse TNF-α ELISA kit*. Pemeriksaan ekspresi ICAM-1 sel endotel jaringan otak dilakukan pada hari ke-3 dan hari ke-9 dengan pewarnaan *imunohistocimia* (IHC).

Hasil: Kadar TNF-α hari-3 setelah infeksi pada kelompok yang diberi ekstrak buah merah (kelompok III, IV, dan V) menunjukan tidak ada perbedaan yang bermakna ($p=0,839$) dibandingkan kelompok yang tidak diberi buah merah tetapi diinfeksi (kelompok VI). Kadar TNF-α hari-9 setelah infeksi pada kelompok yang diberi ekstrak buah merah (kelompok III, IV, dan V) lebih rendah ($p<0,05$) bila dibandingkan kelompok yang tidak diberi buah merah tetapi diinfeksi. Ekspresi ICAM-1 hari-3 dan hari-9 setelah perlakuan pada kelompok yang diberi ekstrak buah merah lebih rendah ($p<0,05$) bila dibandingkan kelompok yang tidak diberi ekstrak buah merah tetapi diinfeksi.

Simpulan: Pemberian ekstrak etanol buah merah (*P conoideus* Lam) dapat menurunkan kadar TNF-α dan ekspresi ICAM-1 pada sel endotel jaringan otak Mencit Swiss setelah diinfeksi *P.berghei*.

Kata Kunci: *Pandanus conoideus* Lam, TNF-α, ekspresi ICAM-1, *Plasmodium berghei*, Mencit Swiss

INTRODUCTION

Malaria represented life threatening disease caused by *Pasmodium* parasite infected human being through the bite of infected mosquitos[1]. The malaria infection by *P. falciparum* might be severe and fatal such as found in cerebral malaria and it contributed to high mortality of all of age groups, especially in the area of malaria endemic population. In addition to the aforementioned causal factor, he condition was also supported by the virulence of the *P. falciparum* capable of invading old, young and erythropoetic mother cells[2].

Parasitic antigen could activate antigen presenting cell (APC) and represented peptide molecule antigen fragment of Glycosylphosphatidylinositol (GPI) with the help of superficial molecule, which was Major Histocompability Complex (MHC) to introduce antigen with the help of T cell receptor (TCR). The interaction represented the activation of cell T so that it could produce various molecules such as cytokine enabling various cells to communicate each other. The cells interaction depended on the contact signal of the T cell receptor (TCR) and Major Histocompability Complex I or II (MHC-I or MHC-II). The *Major Histocompability Complex* (MHC) and T *Cell Receptor* (TCR) were required in the initial stage of T cell activation that the activated cell T proliferated into cell T helper 1 secreting proinflammation cytokine such as *Tumor Necrosis factor-álpha* (TNF-α), IL-1, IL-2, IFN-γ and cell T helper 2 secreting antiinflammation cytokines such as IL-10, IL-5 and IL-4, which could release cytokine spectrum activating the cell T in a cellular response or cytokine and helped cell B differentiate itself into plasma cells that produced antibody[3,4].

The high TNF-α content in cerebrovascular space could cause more severe damage to cerebral tissue and neurological deficit[5]. The protective mechanism against the malaria pathological condition was mediated by cell T helper 2 (CD4+) that released cytokine IL-4, IL-5 and IL-10 representing immuno-suppressant cytockine by inhibiting the activity of the cell T helper 1 (CD4+), which was the TNF-α by interleukin-10, the increase in the IL-10 content could prevent the damage of the cerebral tissue caused by the cerebral malaria. Severe malaria complication was established by the proinflammation cytokine content in the form of the TNF-α in its high content and its pathological effect, but at its low content it served as antiparasite[2].

The production of the TNF-α would increase the expression of brain endothelial cells such as intercellular adhesion molecule-1 (ICAM-1). Subsequently, the ICAM-1 bound Plasmodium falciparum Erythrocyte Membrane Protein-1 (PfEMP-1) on the surface of parasitized red blood cell (pRBC) and caused cytoadherence pRBC of the brain endothelial cells[6,7,8,9]. Various treatments with antimalaria have been developed, but the main problem was the failure because of parasitic resistence to antimalaria medicines. World Health Organization (WHO) recommended the development of herbal medicines as therapy of health problems[10].

Red fruit was one of foods containing active compounds that consisted of total carotenoid 12.000 ppm, total tocopherol 11.000 ppm, betacarotene 700 ppm, alpha tocopherol 500 ppm and vitamin C 25.70 mg. One of the merits of the red fruit that have been proven in vitro, in vivo and in clinical test was â-carotene to prevent degenerative diseases such as stroke, coronary diseases, cancer, uric acid, osteoporosis and it was able to activate cell T helper and antibody[11].

The treatment of the red fruit of falciparum malaria infection could improve the proliferation of lymphocyte cells, especially through cellular and humoral immunity lines that were mediated by the subset of the cell lymphocyte T helper 2 DC4+, considering that the high content of the active compound of the b-carotene in the red fruit ethanol extract was able to interact with and to activate the cell T helper 2 so that the cellular and humoral immunity lines would be activated through the release of various cytokines[11,12].

Betacarotene would react to carotene dioxigenase in intestine to form retinaldyhide and stored in hepar and when it was needed by

cells, it would be dehydrolized by esterase pancreatic enzyme into retinol and bound by retinol binding protein into the cells and entered ketisol and bound by cellular receptor binding protein and dehydrolized by the retinol dehydrogenase and alcohol dehydrogenase into retinal that was subsequently transformed into all-trans-RA with the help of dehydrogenase. The all-trans-RA entered nucleus and bound to RAR so that transcriptional activation of vitamine A responsive gens took place and the vitamine A played the role in regulating the nonspecific immune function and cellular and humoral responses. Additionally, the vitamine A played the role in the development and the differentiation of the subset T helper 1 and T helper 2 to maintain normal antibody against the impact of the T helper 2 so that the T helper suppressed the production of IL-12, TNF-α and IFN-γ[3,13,14]. Additionally, when all of the trans retinoid acid bound the protein peroxisoe prolifertor-activated receptors (PPAR), they could regulate the protein M2 that functioned to activate the phospholipase C and changed phosphatidylinositol 4,5 triphosphate (PIP2) into diacydlycerol (DAG) and D-yo-inositol-14,5 triphosphate (PIP3) that functioned to regulate intracellular ion Ca^{2+} content by binding the receptor iP3 on the reticulum endoplasma surface and stimulated the release of the Ca^{2+} ion from the reticulum endoplasma, while free Ca^{2+} ions could bind calmodulin andactivated calsineurin so that the calcineurin phosphorilized phosphate using nuclear factor activation T and the NFAT became active and entered the nucleus binding the interferon regulation factor 4 and IRF4 would bind the promoter IL-10 that protein-protein anti-inflammation regulation took place between IL-10, IL-4 and IL-5[15,16,17,18,19].

The β-carotene compound represented provitamine A with multiple binds of cis–β-carotene that it was sensitive to oxidation. The process took place in the carbon chain contain multiple binds, the β-carotene functioned to trap oxygen and as antioxidant that effectively bound free radicals if it was situated in partial oxygen 2-21 mmHg and at the concentration of 260 mg[8,11,20].

MATERIALS AND METHODS

The Preparation of Red Fruit Extract

The red fruit (*P conoideus* Lam) was obtained from Wamena, Papua, situated 2.500 m above sea level and it was of 3 months of age, 55 cm of length, 22 cm diameter, 6 kg of weight. It was split and its stem was removed and then the seeds were separated. Subsequently, the seeds were weighted and it gave 2.4 kg and then put into homogenizer and ethanol 70% was added at the ratio of 1:5 (1 kg of the red fruits; 5 liters ethanol). They were soaked for 6 days and stirred three times daily for 2 minutes and covered. After 6 days, the red fruits were blended and filtered using flannel cloth to separate the filtrate and the dregs and then the filtrate was retained in porcelain cup, heated in bath water (70^{o}C) and continuously stirred and aerated till the red fruit extract pasta resulted and was ready to use as experiment material.

The Density of *Plasmodium berghei*

The density of the *P.berghei* in infecting the experiment mice was $1x10^{7}$/0.2ml. The dose was established using inoculum, which was an infected Swiss mouse sacrifized by injecting ketamine 50 mg in 1 ml (IP), while the blood sample was drawn through orbitalis vein in the sum of 10 microliter and liquefied using RPMI-990 microliter and then 0.1 microliter was taken and entered into hemocyto-meter, observed under ray micrsoscope with 100 times magnification till the inocculum was obtained

with the number of parasite of $5x10^7$/mL equivalent to $1x10^7$/0.2mL.

Experimental animal

The samples of the study were male Swiss mice (*Mus musculus* L) of 8 weeks of age, 20-30 g of body weight, which were obtained from LPPT UGM. There were 60 experiment male Swiss mice and 24 mice in reserve that were equally treated in each of the groups. Thus, each of the groups consisted of 10 mice and the total number of the experiment mice was 60 mice. They were randomly assigned to 6 groups, which were KI, KII, KIII, KIV, KV and KVI that gave 10 mice per group. Subsequently, they were adapted for 7 days

The Blood Sampling for TNF—α Conent Examination

On the days 3 and 9 after the infection by *P. berghei,* blood samples were drawn from 5 sacrificed mice of each of the grups through orbitalis vein with microhematocryte. The blood samples were then centrifuged to obtain serum. The resulting serums were then kept in a freezer. Meanwhile, the remaining mice in each of the groups were kept alive for examination. All of the mice serums in the freezer were taken for centrifugation to obtain pure serum that was used for sandwich ELISA preparation examination with mouse TNF-á ELISA test Kit.

The preparation of the detector complex was made by mixing anti-mouse TNF-biotin conjugate and streptovidin alkaline conjugate in the same volume. The preparation of 60 x N µL for each reagent (N was the number of mixture used in the assay). The mixtgure was kept at ambient temperature till it was used. It took 60-90 minutes from the preparation to the use and then the remaining solution was kept at 2°C to 8°C for 2 months.

The transfer of 150 µL sample and the control in the plate mixture 96 with 2 replications were then covered with adhesive cover carefully to prevent air bubbles in the mixture and adhered to the plate and the cover. Subsequently, it was incubated for an hour at 37°C. The incubation periode might be prolonged to 3 hours to increase the OD value and then the cover was carefully opened, all of the mixture were aspired, 300 µL washing buffer was added to each of the mixtgures and aspired again. The washing was repeated twice and then the results were aspired once again to clean all of the remaining washing buffers. Subsequently, 100 µL detector complex was added to each of the mixtures carefully to prevent contact with the mixtures.

The plate was covered using adhesive cover and incubated for an hour at 37°C. Close to the end of the incubation period, 2mL substrate buffer solution was immediately added to 20mL with akuades. Two tablets of substrates were dissolved using sterile pincers into the buffer in vortex mixer. Make sure that the substrate has been homogeneously dissolved. The substrate pNPP must be kept in darkness or covered using aluminum foil and used in 30 minutes.

The plates taken from the incubator were used to repeat the wasching procedure and 100 µL sustrate pNPP was added to each of the mixtures and covered using adhesive cover and incubated at 30°C while avoiding the exposure to any lamp or covered using aluminum foil.

After 30 minutes incubation and in the interval of 30 minutes, monitoring must be conducted for the development of colors and the complete removal of the air bubbles before the monitoring. The absorbance value at the 450 nm for 7000pg/mL standard must be between 1.0-2.0 absorbance units before final readings were established. If the value has been reached, the reaction was stopped by adding 30 µL NaOH3M to each of the mixtures. After that, spectrophotometer reading was conducted at the absorbance of 450nm[24].

The Examination of ICAM-1 Expression in Brain Endothelial Cells Using Immunohisto-chemistry (IHC) Method

On the day 9 (H_9) there were six mice taken from each of the groups sacrificed for brain endothelial cell isolation of the Swiss mice infected by *P. berghei* and given the red fruit extract using immunohistochemistry (IHC). The ICAM-1 expression was observed using ICH ICAM-1 test Kit.

The cells were washed using PBS pH 7.4 three times for five minutes. They were given 0.02% sodium aside drops. The tissue might be kept in freezer for several days. They were washed using PBS pH 7.4 three times for five minutes. They were given H_20_2 drops in the PBS for 10 minutes. They were given blocking serum 5% PBS containing Triton-X 0.25% for an hour. They were washed using PBS. The primary antibody ICAM-1 was incubated in serum 1:200 for 24 hours. The tissue was kept at 4°C and then removed and kept at ambient temperature for 15 minutes. The tissue was washed using PBS twice for five minutes. The cells were incubated using secondary antibody biotin-goat-antimouse 1:400 twice for five minutes. They were given diamino benzidine (DAB) drops in DAB buffer. They were given courstexin drops for 10 minutes. It tissue was washed using akuades for 10 minutes. It was aerted at ambient temperature and covered using glass object. After that, it was aerated overnight and observed under microscope at 200 times magnification[25].

The percentage of the expression of the inter-cellular adhesion molecule-1 (ICAM-1) of the brain endothelial cells was established by calculating the number of the cells in the expression of the intercellular adhesion molecule-1 (ICAM-1) for each of the brain endothelial cells in each of the preparations and multiplied by 100%.

The expression of ICAM-1 of the brain endothelial cells:

$$= \frac{\text{The Number of ICAM-1}}{\text{The Total Number of Cells}} \text{ X } 100\%$$

RESULTS AND DISCUSSION

The red fruit (*P conoideus* Lam) ethanol extract was given for 14 days before the Swiss mice was infected by *P.berghei* and continued to the period after the infection.

Table. 1 The mean TNF-α contend of the Swiss mice on the days 3 and 9 after the infection by *P. berghei*

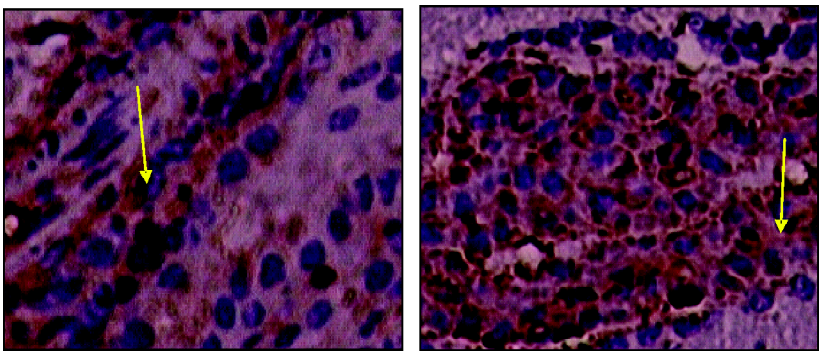
Group	The Mean TNF-α Content after Infection (ng/ml)	
n=3	Day 3	Day 9
I. With no BM, not infected by *P. berghei*	0.009 ± 0.006	0.007 ± 0.002
II. With BM 260 mg/kgbw, not infected by *P. berghei*	0.008 ± 0.003	0.013 ± 0.006
III. With BM 130 mg/kgbw, infected by *P. berghei*	0.007 ± 0.001	0.015 ± 0.005*
IV. With BM 260 mg/kgbw, infected by *P. berghei*	0.010 ± 0.007	0.015 ± 0*
V. With BM 520 mg/kgbw, infected by *P. berghei*	0.009 ± 0.002	0.,012 ± 0.002*
VI. With no BM, infected by *P. berghei*	0.010 ± 0.003	0.026 ± 0.003

*Note: BM = Red fruit ethanol extract; * = P<0,05 (as compared to group VI)*

Table 2. The mean ICAM-1 expression in the brain endothelial cells of the Swiss mice on the days 3 and 9 after infection by *P. berghei*

Group	(ICAM-1 Expression/Number) Cell 100	
n=3	Day 3	Day 9
I. With no BM, not infected by *P. berghei*	0.319 ± 0.168*	0.878 ± 0.133*
II. With BM 260 mg/kgbw, not infected by *P. berghei*	0.307 ± 0.532	9.052 ± 2.333
III. With BM 130 mg/kgbw, infected by *P. berghei*	7.681 ± 6.692*	19.204 ± 5.513*
IV. With BM 260 mg/kgbw, infected by *P. berghei*	7.068 ± 4.021*	2.944 ± 1.775*
V. With BM 520 mg/kgbw, infected by *P. berghei*	27.274 ± 14.580	16.719 ± 11.975
VI. With no BM, infected by *P. berghei*	26.980 ± 4.087	27.373 ± 0.102

*Note: BM = Red fruit ethanol extract; * = P<0,05 (as compared to group VI)*



A B

Figure 1. Histological description of the brain endothelial cells of the Swiss mice after the infection by *P.berghei* (a) On the day 3, (b) On the day 9 (immunohistochemically stained, 200 x magnifications) Arrows indicated ICAM-1 expression

The Impact of the Treatment of the Red Fruit Extract on the TNF-α Content

Group II was given the red fruit extract at the dose of 260 mg/kgbw, but not infected by *P. berghei* and indicated the increase in the TNF-α content (0 013±0 006) because the red fruit contained β-carotene or provitamin A that were capable of regulating immune function, played an important role in the development and the differentiation of the subset Th1 that produced proinflammation cytokine such as TNF-α and Th2 that subsequently produced anti-inflammation cytokine to keep the antibody normal. It was also the case of the cytokine capable of reacting to non-specific and specific immune systems[3,14].

The TNF-α content on the day 9 after the infection by *P. berghei* of the groups III, IV and V given the red fruit ethanol extract at the doses of 130, 260, 520 mg/kgbw was lower (p<0.05 than that of the group that was not given the red fruit ethanol extract and infected by *P. berghei* (group VI). The bioactive compound of β-caroten of the red fruit extract in the intestine would

react to carotene dioxigenase to form redinal dehyde that was stored in hepar. When it was needed, the redinaldehyde was transformed into retinol and bound by the retinol binding protein to the cells through stimulation by retinoid acid gen 6 (STRA6) in the form of all trans retinol (atRO) that was bound by the cellular retinol binding protein (CRBP) and transformed into alcohol dehydrogenase and atRAL after the transformation by the reginal dehydrogenase (RALDHS) into atRA or all trans retinoid acid that would be active and bound to the peroxisome proliferators-activated receptor (PPAR) capable of regulating protein M2 that functioned to activate PLC, to transform phosphatidyl inositol-biphosphate (PIP2) into D-myo-inositol-1,4,5 triphosphate (IP3) and Diacylglycerol (DAG), IP3 would release calcium Ca^{2+} from the reticulum endoplasmic so that it activate the calmoculin and calcineurin capable of phosphorilizing phosphate to activate the NFAT and to regulate IL-10, while IL-10 worked as inhibitor of the NFkβ so that it could not regulate proinflammation protein-protein such asTNF-α[3,15-19,27].

The Impect of the Treatment of the Red Fruit Extract on the ICAM-1 Expression of Brain Endothelial Cells

The ICAM-1 expression on the days 3 and 9 after the infection by *P. berghei* in the third week of the groups III and IV given the red fruit ethanol extract at the doses of 130, 260 mg/kgbw was lower ($p<0.05$) than that of the group that was not given the red fruit ethanol extract, but infected by *P. berghei* (group VI) so that it could be concluded that the inhibition of the ICAM-1 expression on the days 3 and 9 took place because of the treatment of the red fruit ethanol extract after the infection and it was indicative of the presence of the role played by the compounds of β-caroten, tokoferol, vitamin C that worked as antioxidants to bind free radicals[3,8,14].

Additinally, when the malaria infection took place, the malaria parasite could degrade 20-80% hemoglobine erythrocyte host and acidified and degraded organela in vacuola and hemoglobine process. Enzymes would catalize the detoxification of heme ferriprotoporphyrin IX (FP IX) into hemozin and released free radials. The malaria parasite contained protease aspartate so that it could degrade hemoglobine and decrease glucose by activating phosphoenolpyruvate carboxylase to transform piruvate that produced lactic capable of causing lactic acidosis so that the decrease in the oxygen affinity took place at the concentration of Po_2 2,0-2,8 mmHg. Consequently, the decrease in the Po_2 pressure caused the change in the structure and the function of β-carotene of prooxidant compound into antioxidant that functioned to bind free radicals because the β-carotene served as antioxidant when the pressure was Po2 2-21mmHg and the β-carotene concentration was 260 mg/ml/kgbw so that it acted as antioxidant. Additinally, the activation of the macrophage in the phagocytosis of the malaria parasite and parasitic erythrocyte caused the release of free radicals[8,14,28-32]. The tocopherol content that functioned as phenolic hydrogen donor to neutralize the β-carotene in its prooxidant condition when the supply of the oxygen was sufficient, and the PO_2 was sufficiently high as in the pre-infection condition, after parasitic infection there were many prooxidant or oxyradicals resulted so that the tocopherol functioned as antioxidant[814].

The ICAM-1 expression on the days 3 and 9 for the group V with BM 520 mg/kgbw and infected by *P. berghei* did not indicate any significant decrease as compared to the group VI with no BM, but infected by *P. berghei* ($p>0.05$). It was corroborated by the study[33] suggesting that the concentration of the dose of the red furit extract of more than 0.06875 mg/ml could give toxic effect in addition to the fact[8,20] that the concentration of the β-carotene in the plasma served as anti-inflammation and antioxidant and should not exceed 260 mg/ml/kgbw so that it served as active antioxidant in Po_2 2- 21 mmHg.

CONCLUSION

It could be concluded that The Tumor Necrosis Factor Alpha (TNF-α) content of the Swiss mice (*Mus musculus* L) infected by *P.berghei* given the red fruit (*P conoideus* Lam) ethanol extract at the doses of 130, 260, 520 mg/kgbw was lower than that of the group of the Swiss mice that were not given the red fruit extract. The number of the ICAM-1 expression in the brain endothelial cells of the Swiss mice (*Mus musculus* L) infected by *P. berghei* and given the red fruit (*P conoideus* Lam) ethanol extract at the doses of 130, 260 mg/kgbw was lower than that of the group of the mice that were not given the red fruit ethanol extract. It was necessary to conduct the study of the CXCL9 expression in the brain endhotelial cells with the activation of $_cPLA_2$ that caused cerebral malaria.

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