ANALYZE OF FLAVONOID FROM BEE PROPOLIS WHICH SOURCES IN INDONESIA AS ANTI PLASMODIUM MEDICINE

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INTRODUCTION

MALARIA
HEALTH PROBLEM IN THE WORLD

PARASITES PROTOZOA
GENUS PLASMODIUM

WHO

INFORMATION

2,4 MILLION PEOPLE LIFE IN AREA ENDEMIS
AFRICA 45; AMERICA 21; MEDITERRANIA 14; ASIA 8
EUROPA 4; ASIA PASIFIC 9

INDONESIA

DRUG RESISTANCE TO ANTIMALARIA
Cycle of parasite in the mosquito & Human body

Mosquito Stages:
1. Infective Stage
2. Diagnostic Stage
3. Oocyst
4. Release of sporozoites
5. Ruptured oocyst

Human Liver Stages:
6. Liver cell
7. Infected liver cell

Exo-erythrocytic Cycle:
8. Mosquito takes a blood meal (injects sporozoites)
9. Ruptured schizont
10. Schizont

Sporogonic Cycle:
11. Oocyst
12. Release of sporozoites

Human Blood Stages:
13. Mosquito takes a blood meal (ingests gametocytes)
14. Immature trophozoite (ring stage)
15. Mature trophozoite
16. Exflagellated microgametocyte
17. Microgamete entering macrogamete

Erythrocytic Cycle:
18. Macrogametocyte
19. Gametocytes
20. P. falciparum
21. P. vivax
22. P. ovale
23. P. malariae

Image credit: CDC, http://www.dpd.cdc.gov/dpdx
Mechanism of action of hemozoin and new permease Line (NPP)

Target Potential

Transport membrane parasit

Host protein breakdown

Fraksi flavonoid Source of drug

Fraksi flavonoid bee propolis:

Flavonoid fraction of compounds suspected to have constraints on the degradation of hemoglobin and heme detoxifikasi parasites as well as the parasite membrane inhibitor

The death of the malaria parasite
1. Propolis (bee glue) is a dark-colored resinous substance collected by bees from poplar buds and other plants and used to seal their hives.

2. These compounds can be grouped as follows: free aromatic acids; flavonoids; benzyl, methyl butenyl, phenylethyl, cinnamyl and other esters of these acids; chalcones and dihydrochalcones; terpenoids and others such as sugars, ketones, alcohol
3 Although in small quantities, these compounds can be very important to propolis activity. It has been used in folk medicine since ancient times and it is now known to be a natural medicine with antibacterial, antifungal, antitumoral, antioxidative, immunomodulatory and other beneficial activities.

4. Although there is much research about propolis, the data of propolis for antiplasmodial activity which are mainly related to its immunomodulator effect do not exist. The purpose of this research is to examine the antiplasmodial and immunomodulator effects of Indonesian propolis.
- Propolis was produced by honeybee batu from the apiary located on Malang (East Java, Indonesia). A 70% propolis ethanolic solution was prepared.

- A later week, this solution was filtered and used to prepare a 10% Propolis Hydroalcoholic Solution (PHS).
Animals:

1. Thirty male balb/c mice weighing approximately 25-30 g aged between 6 and 8 weeks old were used for propolis treatment *in vivo*. The mice infected with 0.1 ml of the *P. Berghei* suspension at a concentration of $10^7$ parasites per mouse on day 0.

2. The control group was given the solvent in equal volume for the same duration. During the experimental period, the animals were housed under standard laboratory conditions with adlibitum water and balanced food.
Parasitemia analysis:

- The methods of Giemsa blood smear was used to count the number of the parasites.

- The blood in the periphery was taken from the tails of mice and prepared to a sample of thin and thick smeared blood method with Giemsa coloring. The number of parasitemia was calculated by determining the percentage of red blood cells infected by *P. berghei* in 5000 red blood cells.
1. The method of blood Giemsa blood smear was used to count the number of parasites
2. The Blood in the periphery was taken from the tails of mice and prepared to the sample
3. The Number of Parasitemia was calculated by determining the percentage of RBC by infected P. Berhgei
• The test of non-specific phagocytosis activity was conducted in vitro, in reference to Leijh et al.
• The latex particles were resuspended in PBS to obtain the concentration of 2.5´10^7 mL
• Peritoneum macrophages, cultured a day before, were washed twice in the RPMI medium and then added with latex suspension of 200 μL well-1 and incubated in a CO2 5% incubator, 37°C, for about 60 min
• After that, the cells were washed with PBS 3X to remove the unphagocytosysed particles, dried in the room temperature and fixated with absolute methanol. Once dried, the cells attached to the cover slip were colored with Giemsa 20%. The percentage of cells phagocytizing latex particles and the number of phagocytized latex particles were counted from 100 cells, using a light microscope with the zoom of 400x
RESULT

• From the research results shown on Table 1, we can see that the group of mice PHS-administered in the dosage of 100 mg kg\(^{-1}\) BW has higher IgG concentration than those in the dosage of 25 and 50 mg kg\(^{-1}\) BW. With regards to the humoral immune response, the ethanolic extract of propolis 500 μg mouse\(^{-1}\) increases the antibody production in Sheep Red Blood Cells (SRBC)-immunized mice.
RESULT

- The phagocitosys response includes the phagocytosis activity (the number of active phagocyt in 100 phagocyt cells) and phagocyt capacity (the number of phagocytized plasmodium in 50 active phagocyt cells. Table 2 shows the PHS effect on the activity and phagocytosis capacity of the macrophages.
DISCUSSION

1. The functional immune response occurs when the parasites undergo asexual erythrocytic phase. As soon as the parasites enter the red-blood cells, the antibody can be detected using conventional serology method. Recent reports indicate that several types of flavonols stimulate human peripheral blood leukocyte proliferation.

2. The loss of these numerous erythrocytes triggers the bone marrow to produce new ones. As the mice were infected by *P. berghei*, the parasitaemia increased since the body immuneresponse was not quite perfect and the parasites were still phagocytosed slowly mainly in lymph. A lot of infected erythrocytes were found in lymph and phagocytosis by macrophage. The phagocytosis on IgG sensitized cells and C3b-attached cells by the lymphatic macrophages of infected mice was higher than that of normal mice. *Plasmodium berghei* is a synchronized parasite target erythrocytes infected by young parasites might not cause the change of erythrocyte membrane surface. Lymph macrophages activated by malarial infection may phagocytose those...
CONCLUSION

Propolis Hydroalcoholic Solution (PHS) showed more immunostimulant activity than antiplasmodial activity, proved by the increase of IgG and the macrofage phagocytosis activity and capacity in the dosages of 10, 25, 50 and 100,500 and 1000 mg kg\(^{-1}\) BW. The antiplasmodial activity of PHS was due to the mice immunity increase so that they lived longer.