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

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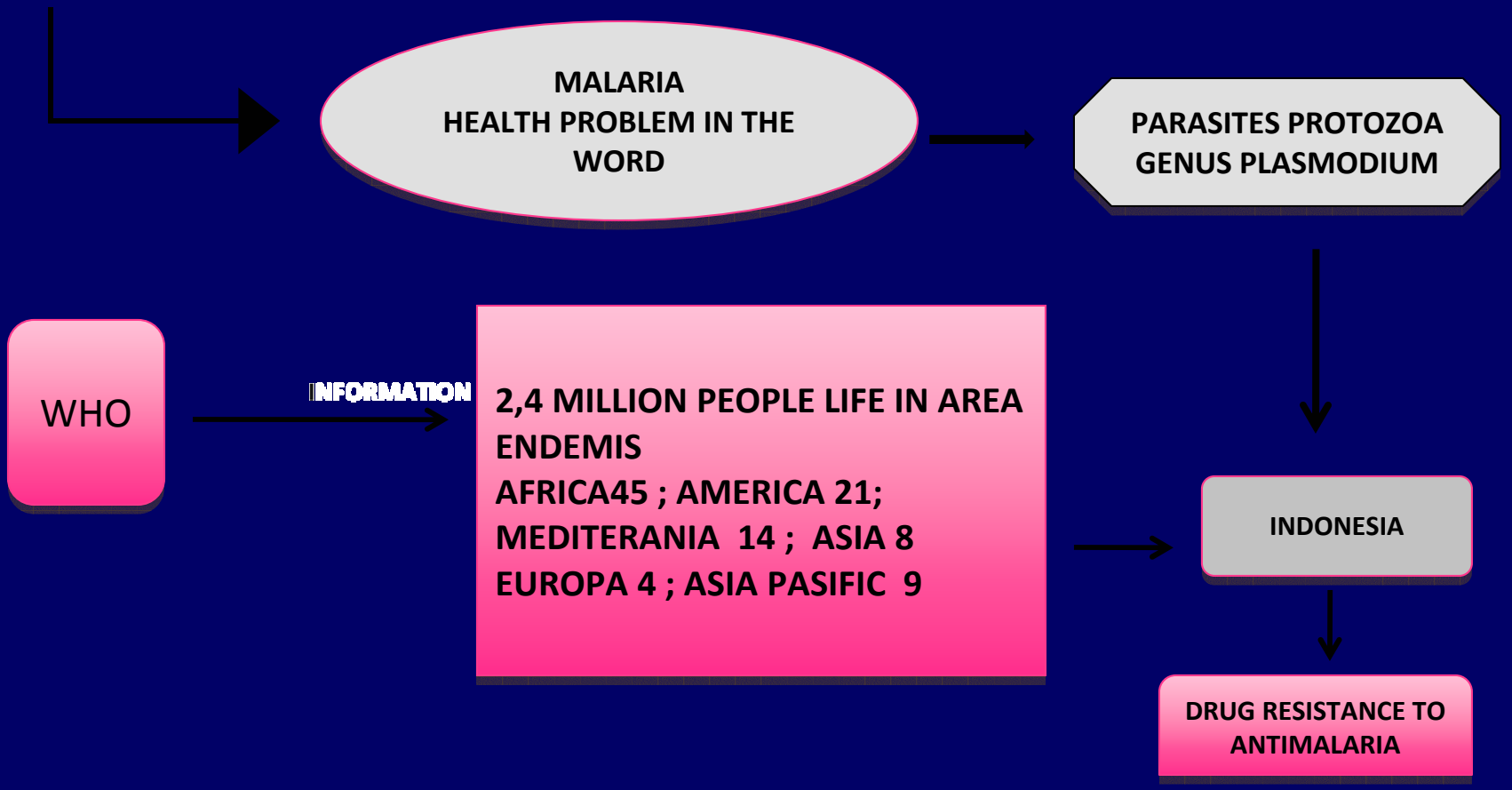
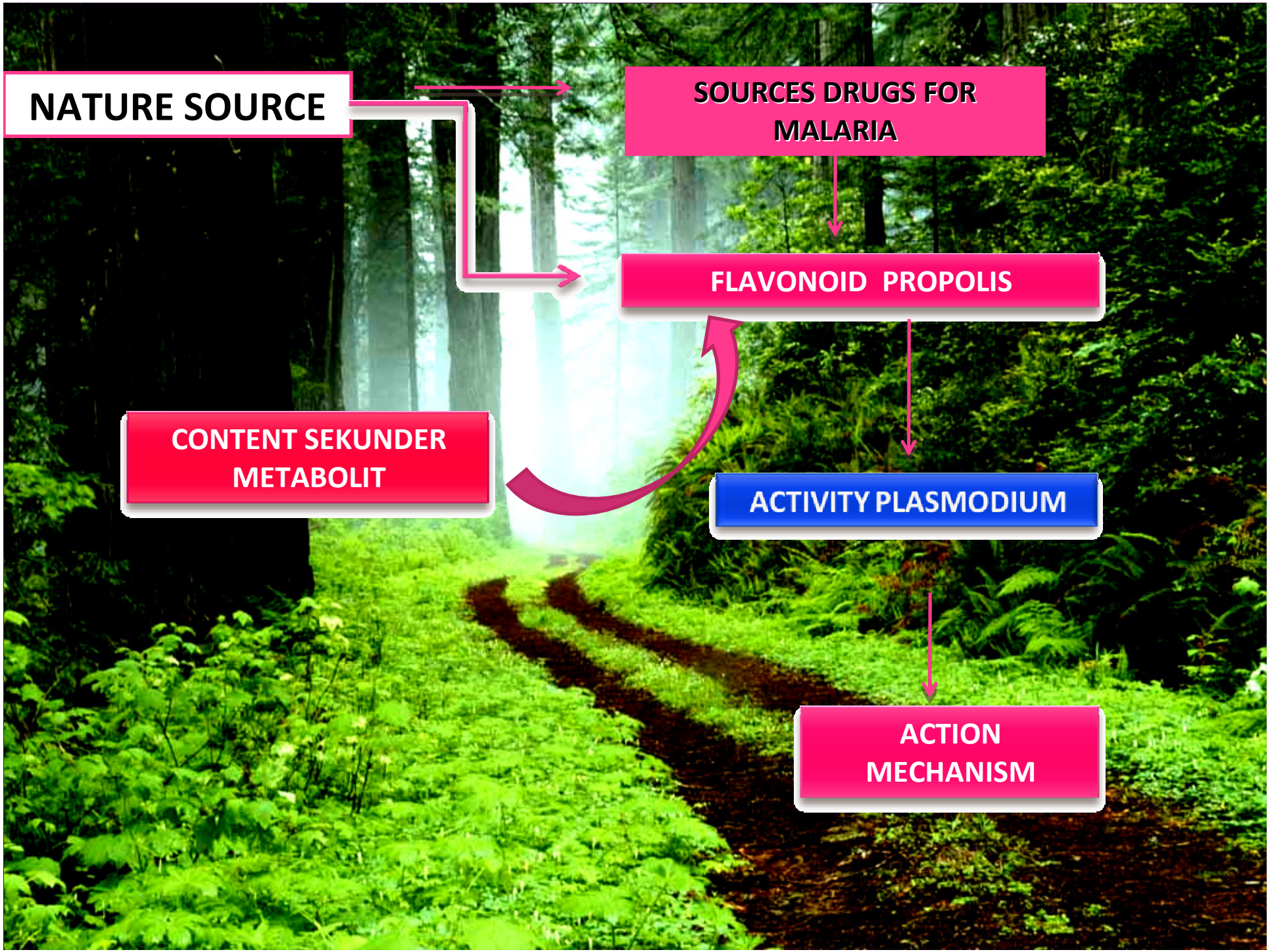
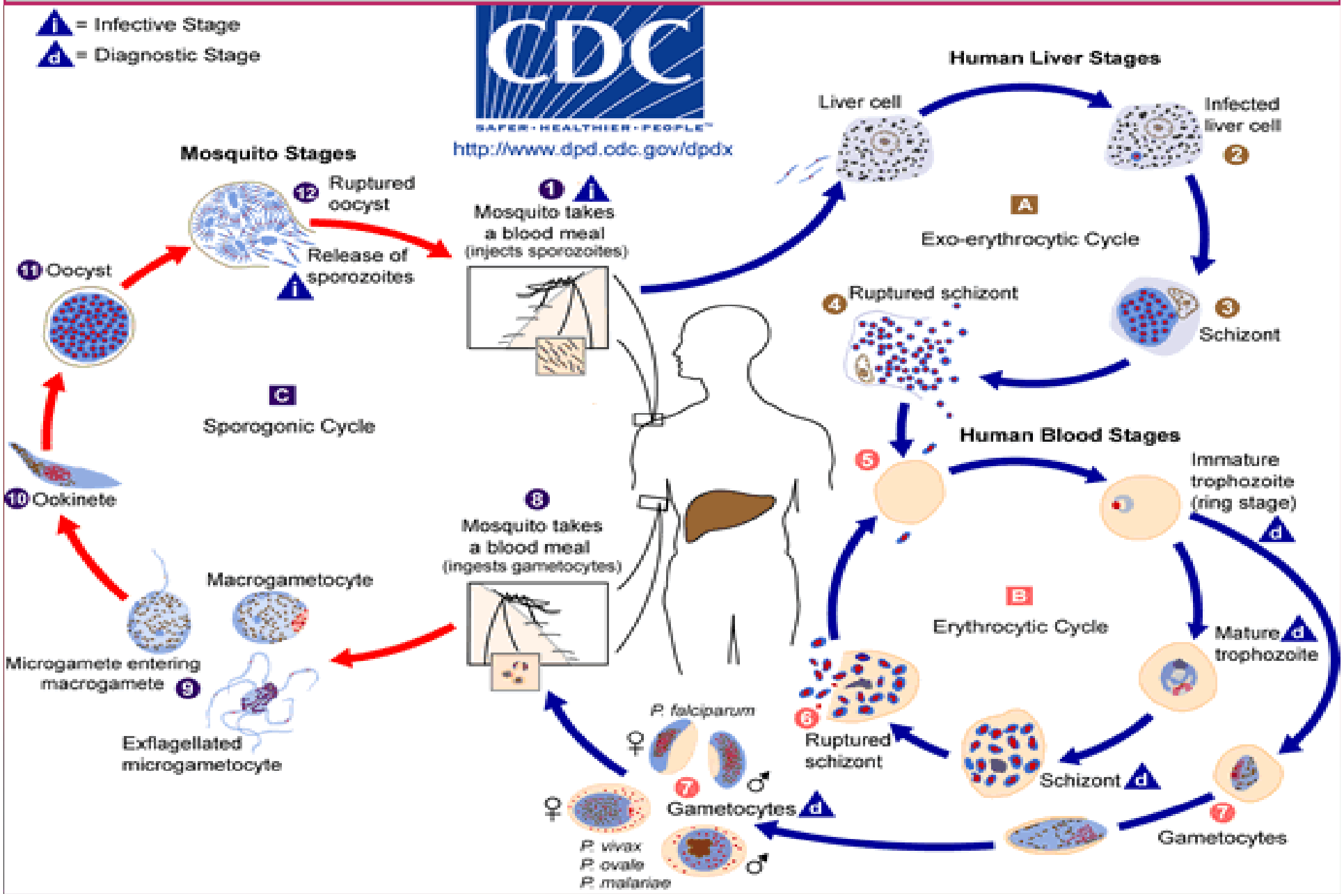
INTRODUCTION

- **BACKGROUND**




NATURE SOURCE
SOURCES DRUGS FOR MALARIA
FLAVONOID PROPOLIS
CONTENT SEKUNDER METABOLIT
ACTIVITY PLASMODIUM
ACTION MECHANISM

Cycle of parasite in the mosquito & Human body



Mechanism of action of hemozoin and new permease Line (NPP)

MALARIA DRUG DISCOVERY

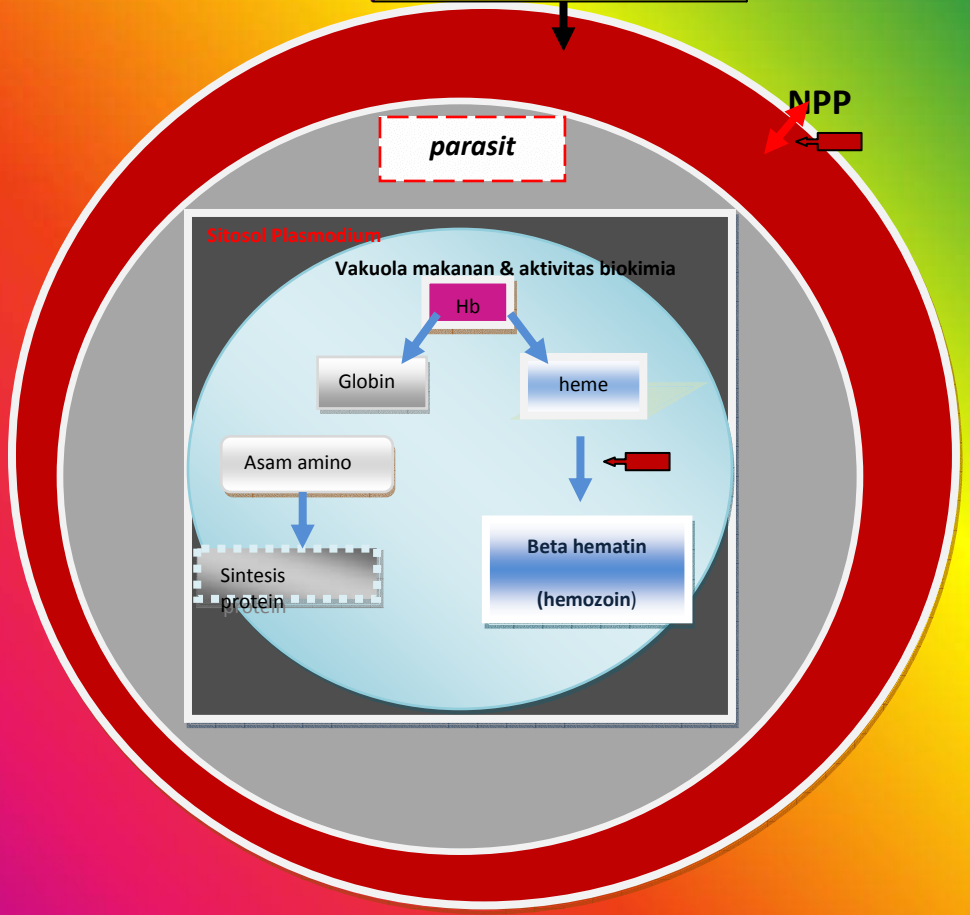
Target Potensial

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 detosifikasi parasites as well as the parasite membrane inhibitor

The death of the malaria parasite

PROPOLIS

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

PROPOLIS

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 its 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

MATERIALS AND METHODS

- 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% PropolisHydroalcoholic 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 solven 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 parasits

- 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. Thenumber of parasitemia was calculated by determining the percentage of red blood cells infected by *P. berghei* in 5000 red blood cells.

Technique Painted with Giemsa

1. The metode of blood Giemsa blod 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 persentage of RBC by Infected P. *Berhgei*

Analisys Macrophage

- The test of non-specific phagocytosys 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´107 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 un phagocytosysed 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 erythrocitic phase. As soon as the parasites enter the red-blood cells, the antibody canbe detected using conventional serology method. Recent reports indicate that several types of flavonols stimulate human peripheral blood leukocyte proliferation**
2. **The lost ofthese numerous erytrocytes trigger sthebone marrow toproduce new ones.As the mice were infected by *P.berghei*,the parasitaemia increase since the body immuneresponse was not quite perfect and the parasites werestill phagocytosized slowly mainly in lymph. A lot ofi nfected erythrocytes were found in lymph and phagocytosis by macrofage. The phagocytosis on IgG sensitized cells and C3b-attached cells by the lymphatic macrofages 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 macrofages activated by malarial infection may phagocytosize those**

CONCLUSION

Propolis Hydroalcoholic Solution (PHS) showedmore 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.