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## The Effect of Phycocyanin on Caspase-3 Trophoblast Cells Models in Rats Preeclampsia Inducible Interleukin 6 (IL-6)

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### ABSTRACT

Preeclampsia is a pregnancy pathological condition characterized by increased blood tekanan and the occurrence of Uriah protein, which can be caused by inadequate trophoblast invasion and spiral arteries remodeling failure will be the cause of death Fetomaternal. In preeclampsia increased IL-6 in the trophoblast cells act as a pro-inflammatory mediator with a predominance of Th1 immunology. Interleukin-6 together with TGF beta 1 (Transforming Growth Factor - beta 1) through 3 STAT pathway stimulates increased secretion of IL-17. Increased levels of IL-17 will further induce apoptosis of endothelial tissue by activating caspase-3. Preeclampsia until now there has been no proper medical treatment, because preeclampsia many factors that can trigger. Several studies have proven Phycocyanin related properties have the effect imunoregulasi namely by stimulating various immune functions such as production of cytokines, chemokines and other anti-inflammatory mediators, the activity of NK cell, B cell antibody production and proliferation of T cells is a laboratory experimental study using research design posttest control group design with treatment by subject menggunakan pregnant rat model of preeclampsia induced by IL-6. A total of 30 female rats. Test Phycocyanin effects on pregnancy pathological models, divided by 6 treatment groups the negative control group, positive control treatment with induction of IL-6, the group P1, P2, P3, and P4 that coadministration treatment groups between Phycocyanin suspension doses 10; 20; 40; 80 / 100gram BB for 5 days. Caspase-3 examination conducted by immunofluorescence method by comparing the expression of Caspase-3 between the control group and the treatment group. In granting Phycocyanin treatment with different doses showed decreased levels of Caspase-3 obtained at the lowest dose use Phycocyanin of 40ng / 100gram BB rats (592.24 ± 19.40). Phycocyanin in its use for the treatment of preeclampsia needed further research, in vivo and clinical.

**KEYWORDS:** Rat Preeclampsia, trophoblast, Phycocyanin, Interleukin-6, caspase-3

### INTRODUCTION

Hypertension in pregnancy, including preeclampsia pregnancy is a pathological condition characterized by increased blood tekanan and the protein Urine<sup>1</sup>. Preeclampsia can be caused by inadequate trophoblast invasion and spiral arteries remodeling failure will be the cause of maternal and fetal mortality<sup>2</sup>. The invasion of trophoblast cells to the lining of the uterus is a process that is important in pregnancy. Trophoblast cells would alter the uterine spiral arteries (spiral arteries remodeling) into the blood vessels that have a low resistance and a large blood flow in the placenta supplies to support the growth and development of the fetus<sup>3</sup>. In pathological pregnancy such as miscarriage, preterm labor, premature rupture of membranes, preeclampsia, IUGR domination Th1 to Th2 immune system in early pregnancy<sup>4</sup>. The dominance of Th1 immune system in pregnancy pathologies will be followed by an increase in proinflammatory cytokine mediators, ie, one of which is the cytokine interleukin-6 (IL-6) is increased in the blood serum, amniotic fluid or the placenta. The activity of IL-6 starting from the bound IL-6 receptor surface IL-6 (IL-6R) and GP 130 (Glukoprotein 130) which in turn activates the Janus Kinase (JAK), lane Signal Transducers and activators of transcription 3 (STAT3), mitogen lines Activated Protein Kinase (MAPK) and will be regarded as a signal to the nucleus to induce transcription of certain target genes<sup>5</sup>. Interleukin-6 together with TGF beta 1 (Transforming Growth Factor - beta 1) through 3 STAT pathway stimulates increased secretion of IL-17. Increased levels of IL-17 will further induce apoptosis of endothelial tissue by activating caspase-3<sup>6</sup>. Preeclampsia until now there has been no proper medical treatment, because preeclampsia many factors that can trigger. Several studies have proven Phycocyanin related properties have the effect imunoregulasi namely by stimulating various immune functions such as production of cytokines, chemokines and other anti-inflammatory mediators, the activity of NK cell, B cell antibody production and T cell proliferation was observed that administration of Phycocyanin 50ng / kg to give effect to the trial imunoregulasi white mouse model of colitis. Spirulina with active ingredients Phycocyanin able to prevent and control the occurrence of preeclampsia, through reduced levels of pro-inflammatory cytokines

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(Th1) of which is IL-6, TGF- $\beta$ , and IFN  $\gamma$ <sup>7,8</sup>. *Phycocyanin* has been clinically researched to prevent and repair the pathological condition of pre-eclampsia. This condition can occur because *Phycocyanin* has the effect of anti-inflammatory and antioxidant<sup>9</sup>. The antioxidant properties of *Phycocyanin* has been demonstrated in various studies in vitro. Using a variety of research methods vary can be concluded that *Phycocyanin* a scavenger protein (neutralizer) were efficient from free radicals and other oxidants react with related pathological conditions such as HCOL and ONOO-<sup>10</sup>.

On the basis of IL-6 has anti-inflammatory properties that result in trigger pathological pregnancy and on the basis *Phycocyanin* antioxidant is necessary to do research related to protective or therapeutic effect of prevention (preventive) of *Phycocyanin* to the pathology of pregnancy to determine caspase-3 in a mouse model of preeclampsia induced Intelleukin- 6.

## MATERIALS AND METHODS

This research is using the laboratory experimental **research design posttest control group design** treatment by the subject of the treatment of the mice. Research using mouse models gravid females with the condition of preeclampsia induced by IL-6. To get the same mice gestational age (homogeneous) then as many as 30 female white rats do estrus cycle synchronization with memperlakuan white rats with Leeboth, Pheromone, Whitten effect. Intelleukin-6 is induced in the day - 10 age pregnant (10 days post mating) via the tail vein for 5 days. Dose IL-6 to make a model of preeclampsia for pregnant rats 5ng / 100 g body weight of rats<sup>11,12</sup>. This study used doses of IL-6 by 5ng / day / 100gram BB rat to create a mouse model of preeclampsia. In each treatment group used 5 animals. On the 6th day (Age bunting day 15) after induction of IL-6 mice were sacrificed for examination caspase-3<sup>13</sup>.

Examination of Caspase-3 was conducted using immunofluorescence. To find Caspase-3 in trophoblasts the examination performed by double staining, these methods do readings using Konvocal Olympus Microscope FX 81. The type and intensity of color recorded will be analyzed using software Imunoflow 7.0 Olympus to obtain the value of the expression of Caspase- 3 on trophoblast. By comparing the value of the expression of Caspase-3 between the control group and the treatment group.

## RESULTS AND DISCUSSION

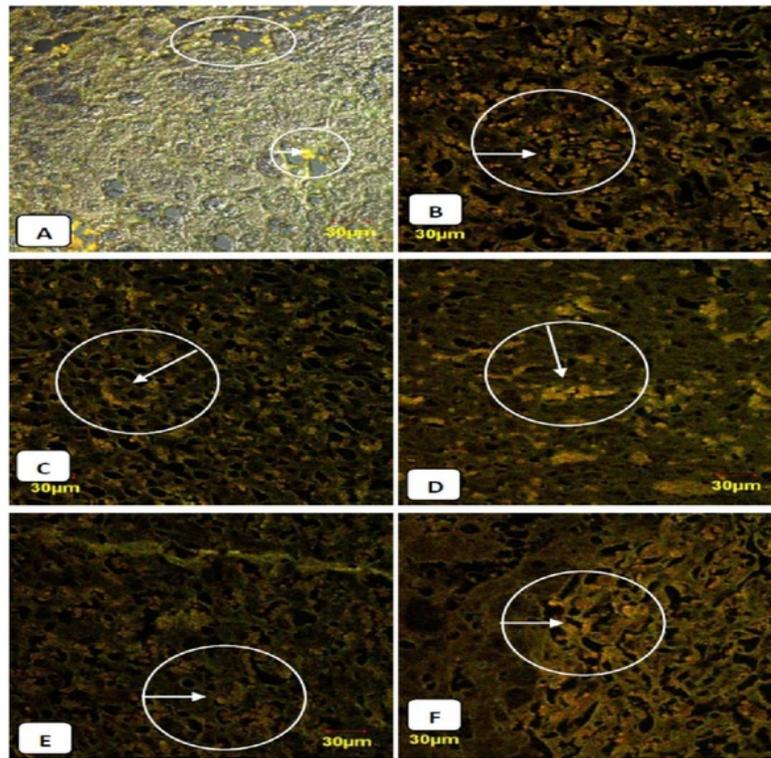
In this study, the induction of IL-6 by 5ng / 100gram BB rats for 5 consecutive days found elevated levels of Caspase-3 was significant in the positive group (803.33  $\pm$  19.40) compared with group therapy treatment. In the study found decreased levels of Caspase-3 after the administration *Phycocyanin*, although it can not reach the levels of Caspase-3 as the negative control group, see Table 1.

**Table 1. Comparison of levels of Caspase-3 in the negative control group, positive control and multilevel dose *Phycocyanin*.**

Mice group Induction of IL-6 5NG / 100gram BB for 5 days	Caspase-3 ( $\bar{x} \pm$ SD)	P
Control (K)	302,77 $\pm$ 16,72a	0,000
positive control	803,33 $\pm$ 19,40b	0,000
Dose <i>Phycocyanin</i> 10 / 100gram BB	720,30 $\pm$ 21,29c	0,000
Dose <i>Phycocyanin</i> 20 / 100gram BB	728,29 $\pm$ 23,04c	0,000
Dose <i>Phycocyanin</i> 40 / 100gram BB	592,24 $\pm$ 19,40d	0,000
Dose <i>Phycocyanin</i> 80 / 100gram BB	621,63 $\pm$ 25,37e	0,000

Description: abc superscript when loading the same letters mean no significant difference, when loading a different letter means having a significant difference between the treatment groups based on comparison test / double comparison Least Significant Difference / LSD (P <0.05).

In granting *Phycocyanin* treatment with different doses showed decreased levels of Caspase-3 obtained at the lowest dose use *Phycocyanin* of 40ng / 100gram BB rats (592.24  $\pm$  19.40), see Fig. 1.



**Figure 1: Pictures of Inspection Results Immunofluorescence Caspase-3 in the rat model of preeclampsia trophoblast research.**

White circle (in no golden yellow color) shows the expression of trophoblast cells "double staining" with Caspase anti 8-3.

Description: A, negative control group; B, positive control group; C, Phycocyanin treatment group with a dose of 10 ng / BB; D, Phycocyanin treatment group with a dose of 20 ng / BB; E, Phycocyanin treatment group with a dose of 40 ng / BB; and F, treatment group with a dose of Phycocyanin 80ng / BB

#### CONCLUSION

*Phycocyanin* in its use for the treatment of preeclampsia needed further research, in vivo and clinical aspects.

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