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Phycocyanin Ameliorate Trophoblast Apoptosis In IL-6-Induced Preeclampsia Rat Models

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ABSTRACT

Preeclampsia/eclampsia is the leading causes in maternal death. At the molecular study, pre-eclampsia caused by inadequate trophoblast invasion to endometrium. However, until now, it has no adequate medical preventive for preeclampsia. It is disease sometimes called as "disease of theory" for lack knowledge in basic molecular pathomechanism. Some study report trophoblast apoptosis is pivotal role in preeclampsia which increased lead to insufficiency trophoblast cell in invasion into endometrium. *Spirulina arthrospira* plant or also called blue-green algae which is in the historical record has been consumed since the days of the Aztec tribe. Several studies have proven that Spirulina have the immunomodulation properties stimulate various immune functions such as production of cytokines, chemokines and other anti-inflammatory mediators. Its active bioactive Phycocyanin (PC) has been shown have an effect as anti-inflammatory and antioxidant and prevent preeclampsia occurrence in rat models through reducing pro-inflammatory cytokines However, there are no information in its role in apoptotic trophoblast. Hence, this study aim to answer PC role in apoptotic trophoblast in preeclampsia rat. **Methods.** This research is an experimental laboratory research with post test only group design. We used rat models with preeclampsia pregnant condition induced by IL-6. To get the same gestational age (homogeneous) then as many as 30 female white rats do estrus cycle synchronization. To make a model of preeclampsia of pregnant rats, IL-6 is used in 10 days post mating for 5 days at dose 5 ng/100 g/day body weight intra tail vein Result. PC has proved reducing preeclampsia trophoblast apoptosis in pregnant rats models induced by IL-6 at dose of 40 n/100 kg weight. **Conclusion.** This study confirm that PC has a protective effect on pregnant rats preeclampsia through its inhibition of trophoblast apoptosis.

Keywords: preeclampsia; apoptosis; phycocyanin; IL-6; immunofluorescence; rat trophoblast.

INTRODUCTION

Preeclampsia/eclampsia is the leading causes of maternal death. It was affect 2-8% of pregnancy worldwide¹. At the molecular study, pre-eclampsia caused by inadequate trophoblast invasion and spiral arteries remodeling failure will (Cross, 2006). The invasion of trophoblast cells to the lining of the uterus is a pivotal process in pregnancy. Trophoblast cell alter the uterine spiral arteries (spiral arteries remodeling) into the blood vessels and gave to decrease its resistance and then more blood flow in the placenta to support the growth of the fetus. Extravilous trophoblast cell invasion would change the extracellular matrix (ECM) lead spiral arteries of the uterus dilate towards intervillous space for the mother's blood stream to supply fetal nutrition². In pregnancy preeclampsia, there is dominance of T-helper 1 (Th1) to T-helper 2 (Th2) immune system in early pregnancy. The dominance of Th1 immune system in pregnancy preeclampsia followed by an increase in proinflammatory cytokine mediators, interleukin-6 (IL-6), in blood serum, amniotic fluid and the placenta^{3,4}. In pregnancy, IL-6 has an important role in the

preconception phase, implantation and placental development. IL-6 along with other cytokines and growth factors have pivotal role in controlling morphogenesis and coordination placental trophoblast cell proliferation The activity of IL-6 starting from the bound IL-6 receptor surface IL-6 (IL-6R) and Glukoprotein 130 (GP-130), which activates Janus Kinase (JAK), Signal Transducers and activators of transcription 3 (STAT3), mitogen lines Activated Protein Kinase (MAPK) and will be forwarded as a signal to the nucleus to induce transcription of certain target genes⁵.

However, pre-eclampsia pregnancy until now there has no adequate medical treatment for preeclampsia. It is disease sometimes called as "disease of theory" for lack knowledge in basic molecular pathomechanism. Classic preeclampsia associated with an increase in systolic blood pressure ≥ 140 mmHg, diastolic ≥ 90 mmHg and accompanied by urinary protein. increased IL-6 induced trophoblast apoptosis⁶. Increased IL-6 will act as pro-inflammatory mediator with a predominance of Th1 immunology. Inflammation microenvironment changes

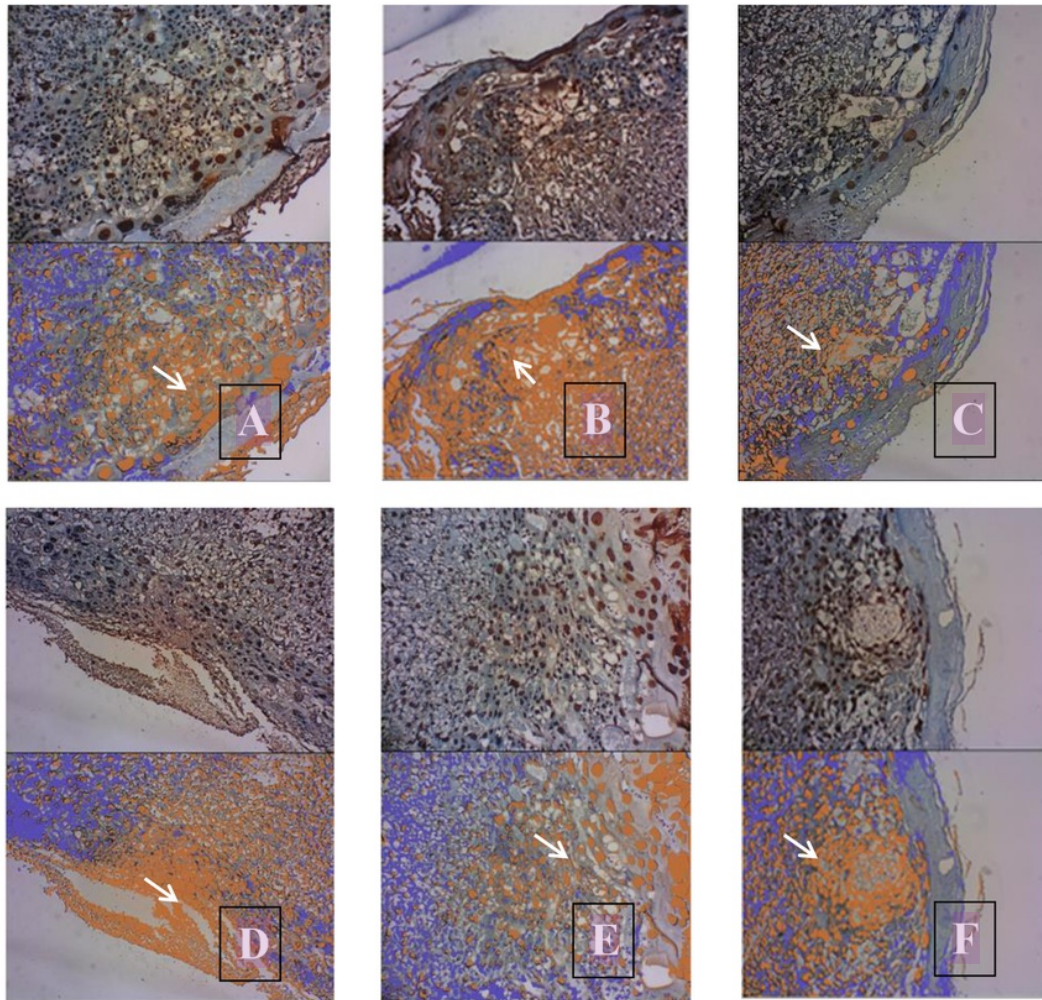


Figure 1: TUNNEL expression in placental trophoblasts. White arrows indicate apoptotic trophoblast area which TUNNEL immunofluorescence stained. (A) Control group; (B) IL-6 group; (C) IL-6 + PC 10 ng/100 bw; D, IL-6 + PC 20 ng/100 bw; E, IL-6 + PC 40 ng/100 bw; and F, IL-6 + PC 80 ng/100 bw.

Table 1: TUNNEL expression shows in all groups.

Group	TUNNEL ($\bar{x} \pm SD$)	P
Control (C)	15,97 \pm 3,39 ^a	0,000
IL-6	78,72 \pm 4,20 ^b	0,000
IL-6 + PC 10/100gram BB	74,79 \pm 4,24 ^b	0,000
IL-6 + PC 20/100gram BB	57,26 \pm 13,34 ^c	0,000
IL-6 + PC 40/100gram BB	48,80 \pm 3,24 ^c	0,000
IL-6 + PC 80/100gram BB	49,96 \pm 8,43 ^c	0,000

lead to characteristics shift of trophoblast cells initially prone to Fas ligand (FasL) become more susceptible to apoptosis³. IL-6 along with Transforming Growth Factor-beta 1 (TGF β -1) through 3 STAT pathway stimulates secretion of IL-17. It will further induce apoptosis of endothelial tissue by activating caspase-3 and increase the ratio of BAX / BCL2⁷. In preeclamptic pregnancies,

apoptosis process increased lead to insufficiency trophoblast cell invasion. Biomolecular pathways associated with the immune system, balance of Th1 and Th2 immune system is now a new trend happening in the pathophysiology of preeclampsia. The dominance system that is pro-inflammatory Th1 second trimester of pregnancy, as evidenced in cases of preeclampsia^{4,8}.

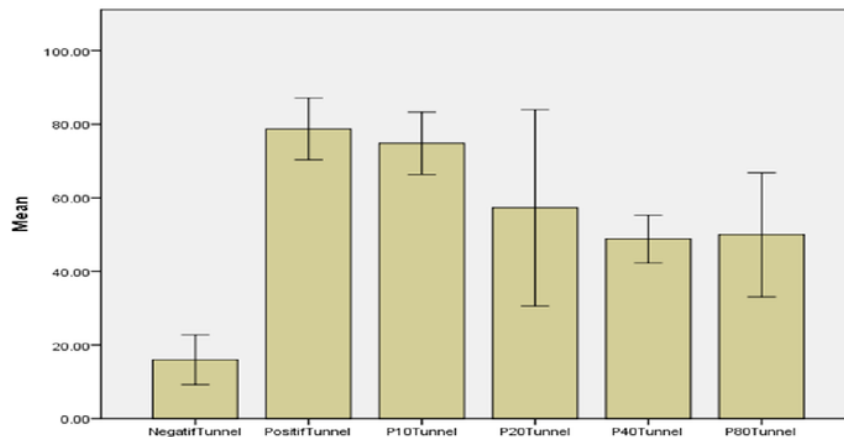


Figure 2: The diagram shows mean compare between group of TUNEL. It shows Negative IL-6 groups, IL-6 groups, and consecutive dose of PC treat group. PC optimum dose 40 ng /100 gram rat weight to decrease TUNEL expression compared to otherdose of PC treat group.

Spirulina arthrospira plant or also called blue-green algae which is in the historical record has been consumed since the days of the Aztec tribe. Several studies have proven that Spirulina have the immunomodulatory properties stimulate various immune functions such as production of cytokines, chemokines and other anti-inflammatory mediators modulate NK cell activity, B-cell antibody production and T-cells proliferation⁹. Its active bioactive Phycocyanin (PC) has been shown have an effect as anti-inflammatory and antioxidant and prevent preeclampsia occurrence in rat models through reducing pro-inflammatory cytokines produced by Th1 through IL-6, TGF- β , and IFN γ ^{10,11}. Phycocyanin is a blue pigment that classified as a protein complex resembles bilirubin. Giving Spirulina 4,5gram/day, in which the content of the active ingredient PC in Spirulina can prevent the occurrence of preeclampsia and lower blood pressure in patients with preeclampsia¹².

Base on that, we conduct a study to dig the role PC in apoptotic trophoblast process in preeclampsia model in pregnant rat. In this study, apoptotic trophoblast quantification will be measured to elucidate the role of PC to ameliorate apoptotic trophoblast.

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MATERIAL AND METHODS

This research is an experimental laboratory research with post test only group design. We used rat models with preeclampsia pregnant condition induced by IL-6. To get the same rat gestational age homogeneous then as many as 30 female white rats do estrus cycle synchronization. To make a model of preeclampsia of pregnant rats, IL-6 is used in 10 days post mating for 5 days at dose 5 ng/100 g/day body weight intra tail vein¹³⁻¹⁵. In each treatment group used 5 repetition. The experimental animals were randomized with a random selection and grouping as follows:

C1: Control group

C2: IL-6 group with no PC treatment

P1: IL-6 group PC dose of 10 ng.

P2: IL-6 group PC dose of 20 ng

P3: IL-6 group PC dose of 40 ng

P4: IL-6 group PC dose of 80 ng

At 6th day (pregnancy age day-15th) after induction, rat sacrificed for data obtain. Endometrium in uterus is cutted off and fixed in formaldehyd 4% solution. Tissue block in parafin section then sliced in 0,5 mm at glass slide to processed further in immunofluoresce and TUNNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) methods (Enogen number 20160302). Apoptotic trophoblast seen in as brown color in the nucleus while the viable trophoblast cells remain seen as purple/green stain nuclei. The expression was measured quantitively by Image J softwar. Data analyse in compare mean and statistically significant if $p < 0,05$.

RESULTS AND DISCUSSION

In this study, the PC has proved reducing preeclamptic trophoblast apoptosis in pregnant rats models induced by IL-6. We use TUNEL cell count and gained an optimum dose PC to decrease apoptotic cell at of 40 n/100 kg weight. Mechanism PC decrease cell apoptosis is the end result of inhibition process of inflammation and oxidative stress which is Correlation PC inhibits apoptosis in research proven, so it can be concluded PC to repair or inhibit inflammatory processes and oxidative stress resulting in preeclampsia trophoblast apoptosis induced by IL-6^{16,17}. PC dose 40ng / 100gram able to repair or reduce apoptosis of trophoblast. PC shown to inhibit IL-6 pathway cascade through the barriers on IL-17, Stat3. On the track proved to PC oxidative stress can increase levels of SOD, so as to suppress or inhibit the process apoptosis through oxidative stress. Barriers to this oksidative inflammation and stress, significantly reduce apoptosis in trophoblast cells, where the variables measured were TUNEL histochemistry found decreased expression^{18,19}. This study validates the use of the PC has a protective effect on pregnant rats preeclampsia.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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