Available online at www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2017; 9(4): 332-336
doi: 10.25258/ijpcr.v9i04.8543

ISSN- 0975 1556

Research Article

Phycocyanin Decrease Trophoblast Il-17 Expression in Preeclamptic Rat Models

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Available Online: 25th April, 2017

ABSTRACT

Preeclampsia/eclampsia (PEE) was the main cause of death in pregnancy. However, until now, this disease has no adequate medical prevention for lack of its basic molecular pathomechanism. In recent years, there are growing number of study has concern trophoblast apoptosis as important trigger. Thropoblast apoptosis has been shown in many report lead to trophoblast failure to invade into endometrial tissue. Invasion failure of trophoblast was characterized with high expression of IL-17 in its tissue. *Spirulina arthrospira* plant or also called blue-green algae has been consumed since by the Aztec tribe. Several studies have proven that this plant 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. Previous study has been shown that this substance has beneficial effect in preeclampsia inhibition in rat models via its inflammatory reducing effect However, there are lack of information concerning its role in trophoblast IL-17. Hence, this study is conduct to reveal its role in IL-17 expression in trophoblast in preeclampsia. Methods. This research used animal models with PE/E pregnant rat. PE/E induced by IL-6 intravein at dose 5 ng/100 g/day body weight. Animals divided in 6 groups of treatment with two groups control and four groups of PC treatment in different dose. After decapitated, uterus tissue processed to view its IL-17 expression using immunofluoresnce Result. This study has proven IL-17 reducing effect of PC in preeclampsia model of pregnant rats induced by IL -6. PC has reducing IL-17 expression significantly in trophoblast tissue of pregnant rats models induced by IL-6 at dose of 40 ng/100 kg weight. Conclusion. This study confirm that PC has a protective effect on pregnant rats preeclampsia through its inhibiton of trophoblast IL-17.

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

INTRODUCTION

Preeclampsia/eclampsia (PEE) was still the leading causes of maternal death. It was affect 2-8% of pregnancy worldwide[1, 2]. However, until now, this disease has no adequate medical prevention for lack of its basic molecular pathomechanism. Recent study report it was caused by inadequate trophoblast invasion and spiral arteries remodeling failure. Whereas invasion of trophoblast cells to the lining wall of the uterus was a pivotal role in fetal nutrition[3]. Trophoblast cell alter the uterine spiral arteries (spiral arteries remodeling) into the blood vessels and decrase its resistance. It will stimulate 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 intervilous space for the mother's blood stream to supply fetal nutrition[4]. In preeclampsia pregnancy, there was domination of T-helper 1 (Th1) to T-helper 2 (Th2) immune system in early pregnancy[5]. Th1 domination of immune system in preeclampsia pregnancy lead toan increase in proinflammatory cytokine mediators, interleukin-6 (IL-6) in blood serum, amniotic fluid and the placenta[6-8]. However, 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 coordinating placental trophoblast cell proliferation[9]. The activity of IL-6 starting from the bound IL-6 receptor surface IL-6 (IL-6R) and glicoprotein 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 genes[10].

However, until now there has no adequate medical treatment for PEE. It is disease sometimes called as "disease of theory" for lack knowledge in basic molecular pathomechanism[11]. Classic PEE associated with an increase in systolic blood pressure ≥ 140mmHg, diastolic ≥ 90mmHg and accompanied by urinary protein. Recent report says that main feature of PEE is increased IL-6 which induce trophoblast apoptosis[12]. IL-6 act as pro-

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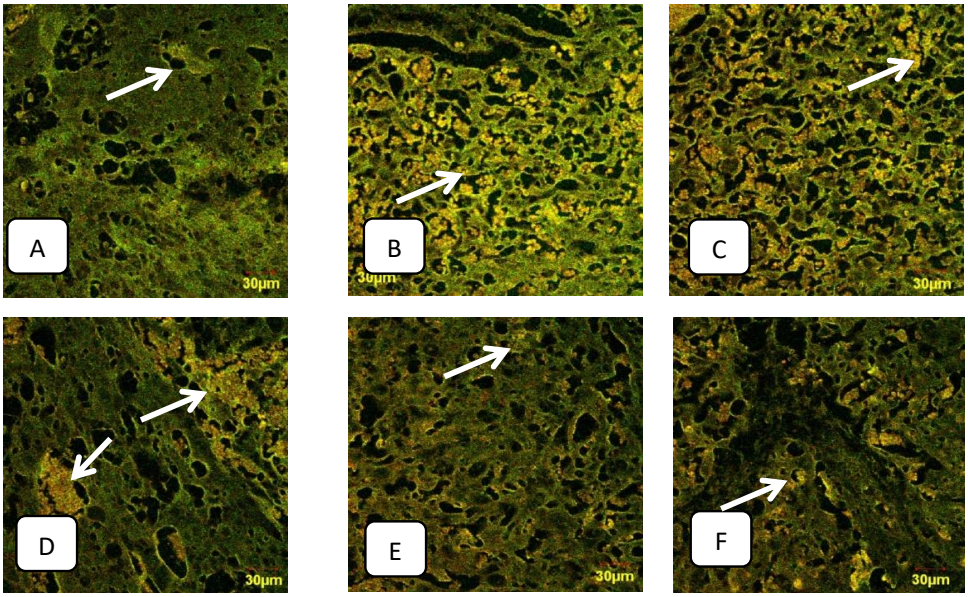


Figure 1: Immunofluorence staining using antibody anti IL-17 in rat trophoblast. White arrow point at yellow show positive immunostaining area. A, negative control; B, IL6 group; C, IL6 group+PC 10 ng/BW group; D, IL6 group+ PC 20ng/BW group ; E, IL6 group+PC 40 ng/BW group ; and F, IL6 group+PC 80ng/BW groups.

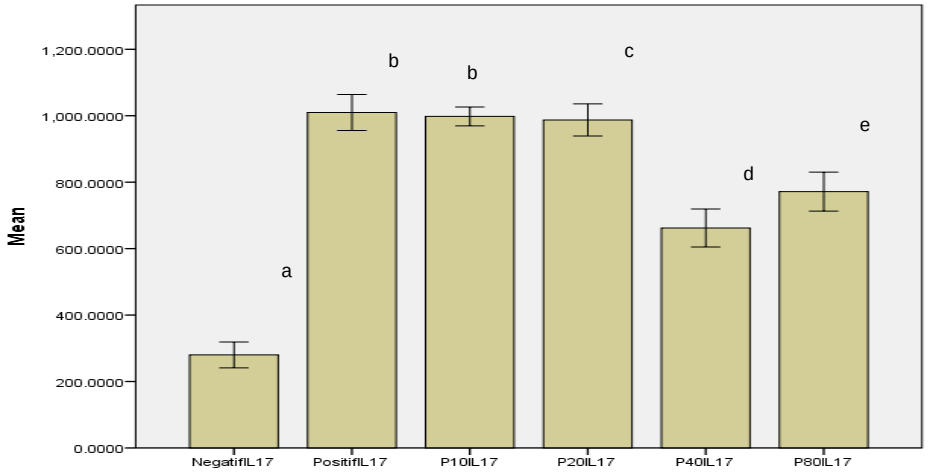


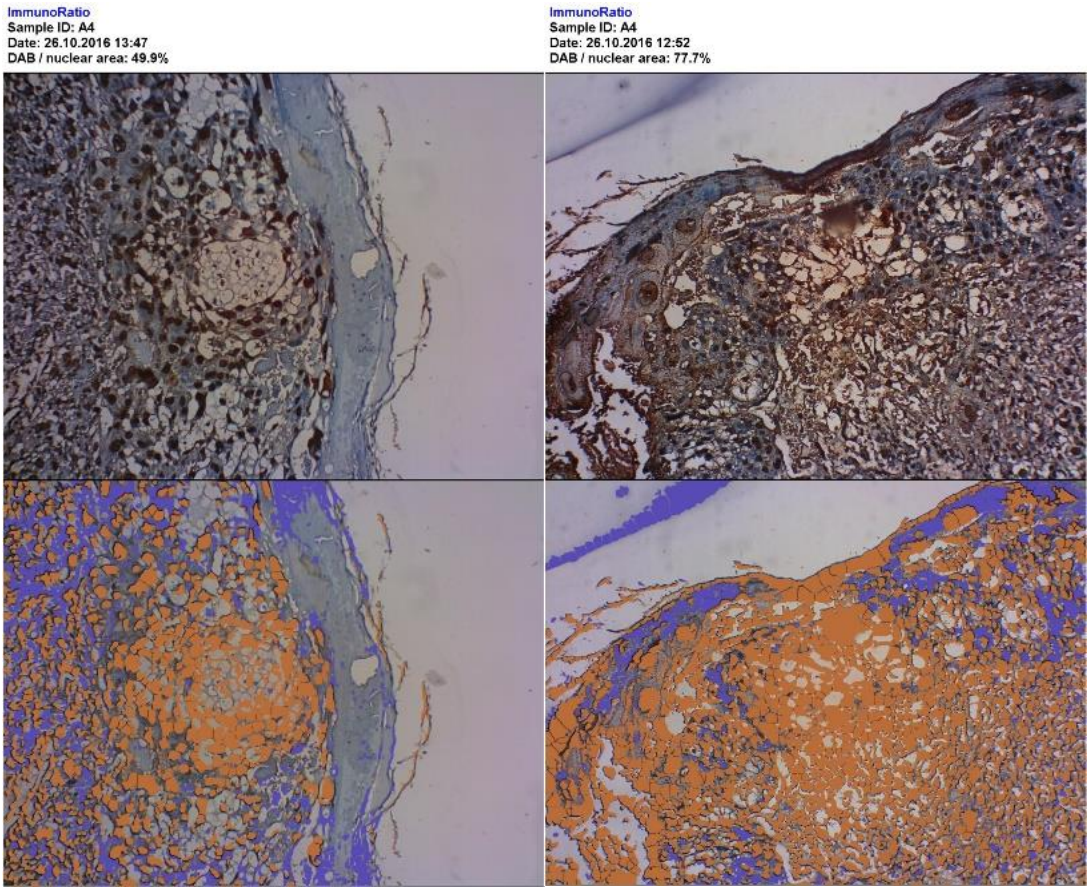
Figure 2: The diagram shows the mean difference IL-17 positive cells. The treatment group was negative, positive and group therapy PC. PC treatment may reduce IL-17 after exposure to IL-6 in mice pregnan. IN ALL There PC dose group the mean decrease in the levels of IL-17 post INDUCED BY IL-6 compared in the positive control group. GROUPS PC dose of 40ng / 100gram BB show reate Most more low average levels of IL-17 compared to other PC group.

Table 3: IL-17 expression has been shown in all groups. Different superscript abc means significant difference using Least Significant Difference/ LSD (p< 0,05).

Groups	IL-17* ($\bar{x}$ ± SD)	p
Control	280,13 ± 19,52[a]	0,000
IL-6	1009,66 ± 27,06[b]	0,000
IL-6 + PC 10/100gram BW	997,98 ± 14,23 [b]	0,000
IL-6 + PC 20/100gram BW	987,35 ± 24,23[b]	0,000
IL-6 + PC 40/100gram BW	661,98 ± 28,62[c]	0,000
IL-6 + PC 80/100gram BW	771,74 ± 29,34[d]	0,000

inflammatory mediator in shifting Th1 predominance immunology. Inflammation microenvironment changes lead to characteristics shift of trophoblast cells initially. It will more prone to Fas ligand (FasL) and become more susceptible to apoptosis[6]. IL-6 along with Transforming Growth Factor - beta 1 (TGFβ-1) through 3 STAT pathway stimulates secretion of IL-17. Further it will induce apoptosis of endothelial tissue by activating caspase-3 and increase the ratio of BAX / BCL2[13]. Downstream process lead to insufficiency trophoblast cell invasion. Molecular pathways associated with the immune system disordera and imbalance of Th1 and Th2 immune system lead to trophoblast apoptosis was gain new attraction in PEE research[7, 14].

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 modulate NK cell activity, B-cell antibody production and T-cells proliferation[15]. Its active bioactive 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[16,17]. PC is a blue pigment that classified as a protein complex resembles bilirubin.. Giving Spriulina 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 preeclampsia[18].

Base on that, we conduct a study to dig the role PC in trophoblast IL-17 and its effect in blood pressure in preeclampsia model in pregnant rat. In this study, IL-17 trophoblast quantification will be measured to elucidate the role of PC to clinical trophoblast.

MATERIAL AND METHODS

This research is an experimental laboratory research with post test only group design. We used 30 female rats induced by intravein IL-6 to make preeclampsia pregnant condition. The same rat gestational age homogeneous then all animals undergone estrus cycle synchronization. IL-6 is used in 10 days post mating for 5 days at dose 5 ng/100 g/day body weight intra tail vein[19-21]. In each treatment group used 5 repetitition. 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

RESULTS AND DISCUSSION

Based on the multiple comparison test, IL-17 were significantly different at each negative control, positive control and administration of different doses PC. In the treatment group PC dose of 10 ng /100gram obtained BW levels of IL-17 was (997.98 ± 14.23); PC dose treatment group 20 ng / 100gram obtained BW levels of IL-17 was (987.35 ± 24.23); PC dose treatment group 40ng / 100gram obtained BW levels of IL-17 was (661.98 ± 28.62) and the treatment group PC dose 80ng / 100gram obtained BW levels of IL-17 was (771.74 ± 29.34). PC treatment with different doses showed a decrease of IL-17 that was significantly in each dose start at 40 ng/100 g bw, where the minimum expression of IL-17 obtained at the optimum dose of PC treatment 40ng / 100gram BW rats (661.98 ± 28.62). Positive control group obtained the expression of IL -17 amounted to 1009.66 ± 27.06. In the treatment group PC dose of 40ng / 100gram in this study, we obtained expression of IL -17 to 661.98 ± 28.62. Greater than 40ng / 100gram BB, which in this study at doses of 80ng / 100gram BB obtained a decrease in IL -17 is less good than the dose of 40ng / 100gram, which amounted to 771.74 ± 29.34. Increasing PC dose with larger doses can stimulate a Th1 immune system, triggering an inflammatory process[22, 23] In this study, it has

been proven that PC can reduce levels of IL -17. However, this result need be confirmed in future research[7, 24].

In this study we use IL-6 to mimic preeclampsia model in pregnant rats, because IL-6 known as an important inflammatory cytokine in preclampsia[19-21]. For the first time this study has proven IL-17 has been reduced effect after PC treatment in preeclampsia model of pregnant rats induced by IL -6. IL -17 inhibition result in decrease inflammatory cascade trigerred by IL -6 This result will decreased or reduction in the inflammatory process induced by IL -6. Cascade inhibition via IL -6 in pregnancy are directly related to the improvement of preeclampsia[14,25]. Moreover, decreasing IL-17 will shift immune system domination i.e Th2. In the second trimester of pregnancy it should be more Th2 domination may last for pregnancy in physiological conditions[7,26]. The use PC for preeclampsia proven to reduce levels of IL -6 in pregnancy preeklampsia and improve blood pressure in pregnancy preeclampsia. PC also proven to reduce cytokines and other inflammatory factors, eg PC inhibiting NADPH oxidase[16].

Inflammation inhibition in trophobalas have apoptosis lowering effect in trophoblast cell. However, this result need more variable to be confirmed in next researcg. In the future, PC can be promise as preeclampsia prevention as it can decrease excessive apoptotic trophoblast cells. Preeclampsia encountered toroblas increase the apoptosis in cells[4, 20, 27].

CONCLUSION

In this study, PC has proved reducing IL-17 expression in trophoblast tissue in pregnant rats models induced by IL-6. We use immunofluoresence area cell count to measure PC treatment effect in IL-17 expression. In this study, optimum dose PC to decrease IL-17 expression in trophoblast tissue was 40 ng/100 kg weight. Mechanism PC decrease its expression is inhibition of inflammation process and maybe oxidative stress inhibition. It has been prove that PC treatment correlate with apoptosis level. It confirmed that PC have inflammatory processes inhibitor properties and oxidative stress inhibitor resulting in decrease of trophoblast apoptosis induced by IL-6 as in our previous study[22, 28]. PC dose 40ng / 100gram able to repair or reduce apoptosis of trophoblast. On the track proved to PC oxidative stress can increase levels of SOD, so as to suppress or inhibit the process apotosis through oxidative stress. Barriers to this oksidative inflamation and stress, significantly reduce apoptosis in trophoblast cells, where the variables measured were TUNEL histochemistry found decreased expression[29,30]. This study validates the use of the PC has a protective effect on pregnant rats preeclampsia via IL-17 inhibition in trophoblas tissue .

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ACKNOWLEDGEMENT

The authors would like to thank to dr.Oski Illiandri,M.Kes from Department of Anatomy, School of Medicine, University of Lambung Mangkurat Banjarbaru Kalimantan selatan for his scientific advice and writing assistance

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