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The Effect of Spirulina on Apoptosis (Stored Biology Materials) To Pregnant Rat Wistar in the Second Trimester wich is Induced By IL-6

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Abstract. The pathology of pregnancy is very diverse, one of which is preeclampsia. Preeclampsia is a complication of pregnancy characterized by hypertension with proteinuria and oedema after 20 weeks gestation. The prevalence of preeclampsia in hospitals across Indonesia varies considerably, that is between 5.75%-9.17% and increased by 40% over the last few years worldwide. Several studies had shown that Spirulina has a microscopic filamentous cyanobacterium contains C-phycocyanin which is a substance with potent cancer chemopreventive activity. The purpose of this study was to determine whether there is the influence of Spirulina on the apoptosis of trophoblast cells expression exposed by IL-6 on pregnant Wistar rats at two trimesters. The type of this result used Post Test Only Control Group Design. This study used a sample group of experimental animals white rat comprising an experimental group (treated) and control groups. The independent variables in this study were Spirulina dose, while the dependent variable was the apoptosis of Wistar rat trophoblast cells expression that is pregnant with preeclampsia model. The results of this study showed that Spirulina can decrease Apoptosis sensitiotrophoblast cells expression in rats models of preeclampsia. The highest apoptotic expression average was in the P0 group, the group was given IL-6 (iv) dose of 5 mg/day was 62.380 and the lowest average of apoptotic expression was in the P3 group, the group was given IL-6 (iv) and Spirulina with a dose of 40 mg/day (oral) is 44.260.

Keywords: Preeclampsia, Apoptosis, Trophoblast, IL-6, Sphirulina

I. Introduction

Maternal Mortality Ratio (MMR) is basically indicators of quality and accessibility of health facilities, based on Indonesian Demographic and Health Survey. In Indonesia, the pathology of pregnancy is very diverse, one of them is preeclampsia. The prevalence of preeclampsia in hospitals throughout Indonesia has varied, between 5.75% -9.17% [1]. and increased by 40% over the past few years around the world [2]. Preeclampsia is a complication of pregnancy which is characterized by hypertension with proteinuria and oedema after 20 weeks of pregnancy. [3]. Pathology of preeclampsia is not known yet with certainty [4], but the clinical sign that a specific sign of preeclampsia is hypertension (systolic blood pressure ≥ 140 mmHg, diastolic pressure ≥ 90 mmHg) and proteinuria (300 mg or greater in the urine of 24-hour specimens or protein for creatinine ratio > 0.30) and occur after the 20th week of pregnancy [5]. This condition accompanied by oxidative stress on the placenta can affect the increasing release of syncytiotrophoblast cells and an increase in apoptosis in the maternal circulation [6].

Apoptosis is a mechanism of programmed cell death [7]. Preeclampsia occurs because the mother syndrome, which involves leukocytes and endothelial cells in the systemic inflammatory response, oxidative stress which resulted in maternal syncytiotrophoblast cell apoptosis increased. [6]. In the systemic endothelial damage occurs micro-deposition of particles microvilli syncytiotrophoblast membrane. [8]. Excessive trophoblast cell apoptosis in endothelial damage is a clinical manifestation of preeclampsia [9]. The inflammatory response of leukocytes and endothelial cells also stimulates the



release of proinflammatory cytokines [6], the study said Interleukin-6 (IL-6) is a multifunctional cytokine which was first identified in 1986 [10].

Interleukin-6 (IL-6) is a cytokine with an important role in the inflammatory response and direct the differentiation of T cells to adaptive immunity. Interleukin-6 (IL-6) is widely expressed in the female reproductive tract and muscles gestational, this cytokine was very important to find out whether the pregnancy in a normal state or not [11] of these cytokines also play a role in acute inflammation, chronic or autoimmunity [12], where these cytokines act in controlling uterine capacity and reception at the main decidualizing [13]. In the event of excessive apoptosis, the adaptive immune response will continue and monocytes will be the largest leukocyte population, it can be seen from the increased expression of Interleukin-6 (IL-6) in the uterus, decidua and placenta cells [14].

Spirulina is a microalga that contains 60-70% protein, 15-25% carbohydrates, 6-13% fat, 8-10% mineral fibres with a fairly complete [15]. Spirulina has a microscopic filamentous cyanobacterium, containing C-phycocyanin which is a substance with a potent cancer chemopreventive activity [7]. This substance is able to induce apoptosis by means of fragmenting DNA and condensation nuclei. Another study states that C-phycocyanin from Spirulina has the ability to reduce the regulatory proteins and anti-apoptosis and upregulates proteins proapoptosis [16], also has the function as an antioxidant that can inhibit the development of immunomodulatory [17, 18].

Based on the description above, researchers interested in conducting research into how the effect of spirulina against apoptosis of trophoblast cells are exposed to the Interleukin-6 (IL-6) in pregnant Wistar rats in two trimesters.

2. Experimental Method

This research is an experimental sample that aims to determine the relationship between the administration of spirulina to the percentage of trophoblast cell apoptosis. The design used in this study is the Post Test Only Control Group Design. This research was designed using a sample of white mouse experimental animal groups consisting of the experimental group (treatment) and the control group. The group is considered equal before the commission of [19].

P0 = IL6 treatment group dose of 5 mg / day

P1 = IL6 and Spirulina treatment group with a dose of 10 mg / day (oral)

P2 = IL6 and Spirulina treatment group with a dose of 20 mg / day (oral)

P3 = IL6 and Spirulina treatment group with a dose of 40 mg / day (oral)

On the 14th day all groups of rats both control and treatment groups were taken placenta then made placental preparations of rats and stained by immunofluorescence method and then observed with confocal microscope. In this study used data analysis using normality test data with *kolmogorov-smirnov test* and test homogeneity of data with *Levene's Test* and then *One Way Anova*

3. Results and Discussion

The occurrence of apoptosis in the white rat placenta was measured by the TUNEL method while the apoptosis mechanism was analyzed by immunofluorescence method using a confocal microscope showing that the administration of spirulina at a dose of 40 mg / day was the most effective dose to reduce apoptosis in rat placenta.

Table 1. Expression of Apoptosis Flocking

Apoptosis	N	Mean	Std. Deviation
Control	5	46,32	6,08169
P0	5	62,38	13,280
P1	5	52,96	2,380
P2	5	62,1	12,421
P3	5	44,26	9,416

Based on above the table 1 it can be seen that the highest average apoptosis expression was in P0 group, the group that was only given IL-6 (iv) the dose of 5 mg/day was 62.380 and the lowest average apoptosis expression was in the P3 group, the group gave IL-6 (iv) and Spirulina with a dose of 40 mg/day (oral) is 44.260.

3.1. Effect of IL-6 induction on trophoblast cell apoptosis

The results of the study showed that the induction of Interleukin-6 (IL-6) was able to increase apoptotic expression. This can be seen from the average control group apoptosis expression which is 46.32 lower ($p < \alpha$) when compared to the positive control group (group with IL-6 treatment (i.v) dose 5 mg/day) which is 62.38.

These results are consistent with the theory that Interleukin-6 (IL-6) will increase its activity through increased leukocytes and Th1 activity which begins with malformation of the maternal immune system to fetoplacental [20] due to Interleukin-6 (IL-6) also has the function of regulating immune response and inflammation [21], so there is endothelial cell dysfunction which is the pathology of preeclampsia resulting from IL-6 function which also interferes with endothelial cell function by increasing blood plasma levels and disrupting regulation of the immune system alone [22]

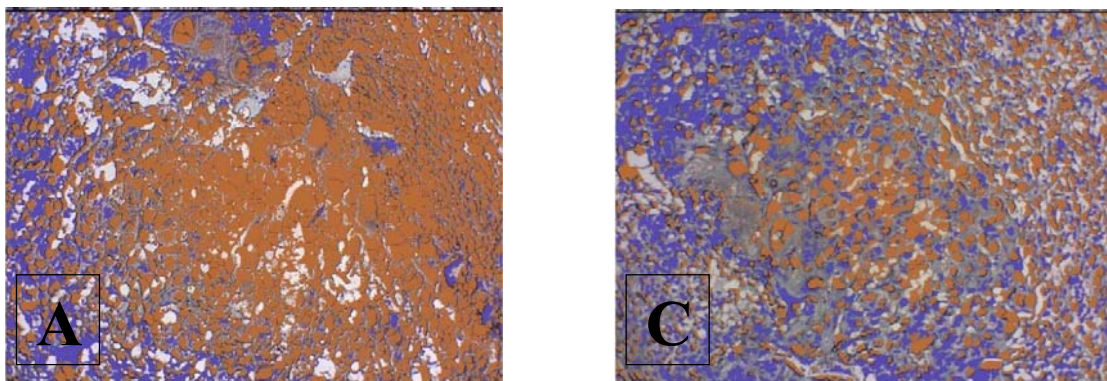


Figure 1 Immunofluorescence staining results. A = shows apoptotic cells after treatment positive control IL-6 seen from red circulation. C = indicates apoptotic cells after treatment Spirulina dose of 40mg / day, circulation the red less than A.

From the results of the study using the Terminal Deoxyribonucleotidyl Transferase dUTP Nick end Labeling (TUNEL) method and then calculating with the Convocal microscope seen from the red fluorescent luminescence, obtained the picture above (Figure 1), found an increase in apoptosis of trophoblast cells shown in figure A with the positive control of IL-6, the results of trophoblast cell apoptosis were 62%. Figure C is post spirulina treatment, found a decrease in apoptosis of trophoblast cells with Spirulina dose of 40 mg / day, obtaining trophoblast cell apoptosis by 36.9%. These results prove that Spirulina is able to reduce trophoblast cell apoptosis.

3.2. Effect of Spirulina on trophoblast cell apoptosis induced by IL-6

3.2.1 Effect of Positive Control Groups and Spirulina Post

The results of the study showed that the administration of Spirulina at a dose of 40 mg/day was able to reduce significant apoptosis ($p < \alpha$) which was 44.26 when compared with the positive control group which was only given a dose of IL-6 (i.v) which was 62.38.

These results are consistent with the theory that Spirulina plants allow the immune system to fight infection. This algae also contains gamma-linolenic acid (GLA), and also provides alpha-linolenic acid (ALA), linolenic acid (LA), stearidonic acid (SDA), eicosapentaenoic (EPA), docosahexaenoic acid

(DHA), arachidonic acid (AA). Vitamins contained in it are vitamins B1, B2, B3, B6, B9, B12, Vitamin C, Vitamin D and Vitamin E. In addition to the above as well as a source of potassium, calcium, chromium, copper, iron, magnesium, manganese, phosphorus, selenium, sodium, and zinc are used by the general public to maintain a healthy body, especially its C-Fikosianin content which is referred to as a substance with potent cancer chemopreventive activity [7, 16] so as to be able to prevent and control the occurrence of preeclampsia, through decreasing levels of pro-inflammatory cytokines [23].

3.2.2. Post Spirulina Dose 10, Dose 20, Dose 40

At P1 and P2 dose have a significant difference, namely the dose of P1 with an average value of expressions 52,960 and P2 with an average value of expression 62,100. It has a significant difference because it was reported from Keman's (2013) study that overexpression of Bcl-2 (B-cell lymphoma 2) will protect lymphocyte cells from apoptosis.

Statistical results of P2 and P3 values do not have a significant difference, namely the dose of P2 with an average value of 62,100 and P3 with an average value of expression 44,260. These results can show that if there is a significant decrease in the level of Interleukin-6 (IL-6) it will be accompanied by a decrease in apoptosis due to the spirulina diet containing C-phycocyanin [24].

While the P1 and P3 doses also did not have a statistically significant difference, P1 with an average value of expressions 52.960 and P3 with an average value of expression of 44.260. This result is the same as the theory produced by David (2010) and Gondo, Harry K (2017) that significantly decreasing Interleukin-6 (IL-6) will be followed by a decrease in apoptosis in trophoblast cells, because the Spirulina diet is mainly C- Phycocyanin can reduce trophoblast cell apoptosis through a reduction in the level of proinflammatory cytokines.

3.2.3. The effective dose of Spirulina in statistics

In this study, Spirulina was given in 3 storey doses of 10 ml, 20 ml, and 40 ml. of the three doses of spirulina given, the effective dose to reduce trophoblast cell apoptosis is at a dose of 10 ml.

The statistical test shows that P1 does not have a significant difference with P3. So the effective dose P1 because at a dose of 10 mg/day can reduce apoptosis to a value of 52.960 while P3 given IL-6 (i.v) and Spirulina at a dose of 40 mg/day (oral) can reduce apoptosis up to a value of 44.260. This result is in accordance with Gondo's research, Harry K (2017) that Spirulina is able to prevent and control the occurrence of preeclampsia, through a decrease in the level of pro-inflammatory cytokines. And several studies have shown that Spirulina has immunomodulating properties that stimulate various immune functions such as cytokine production, chemokines and other anti-inflammatory mediators for protection in pregnant mouse preeclampsia in inhibition of trophoblast cell apoptosis processes.

4. Conclusion

Based on the research that has been done, some conclusions which are: 1, Indications of Interleukin-6 (IL-6) are able to reduce apoptosis which is evident at an average P3 of 44.280 and control (K) of 231.60 and indicates a significant difference; 2, It is proven that Spirulina can reduce apoptosis. With an effective dose of Spirulina which can improve trophoblasts in pregnant Wistar (*Rattus Norvegicus*) rats induced by interleukin-6 (IL-6) is Spirulina dose 40 mg/day orally. In future, the development of similar research can be done with a longer Spirulina treatment period, so that it can be obtained an overview of Spirulina to decrease apoptosis in trophoblasts of Wistar rats exposed to Interleukin-6 (IL-6).

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