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Original Article

The Effect of Spirulina on Apoptosis Through the Caspase-3 Pathway in a Preeclamptic Wistar Rat Model

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Abstract

Objective: To determine the effects of spirulina expression on trophoblast cell apoptosis and caspase-3 expression in a preeclamptic wistar rat model. The preeclampsia model was administered to pregnant rats by interleukin-6 induction. **Materials and Methods:** This research is a Post Test Only Control Group Design. A total of 25 rats with preeclampsia model were divided into five groups, namely negative control group, positive control group, treatment group with IL-6 administration, and spirulina with 10 mg, 20 mg, and 40 mg/100 g body weight. Research has shown that spirulina reduces trophoblast cell expression through apoptosis in a rat model of preeclampsia. The positive control group showed the highest mean expression of apoptosis (62.38) and caspase-3 (804.33. The lowest average expression of apoptosis was 44.26, with 592.24 expressions of caspase-3 in group P3, Spirulina treatment at a dose of 40mg/day /kgBW **Conclusion:** Spirulina inhibits trophoblast apoptosis in preeclampsia through caspase-3 signaling.

Keywords: Apoptosis, caspase-3, interleukin-6, preeclampsia, spirulina, trophoblast.

Introduction

The risk of death from preeclampsia is about 300 times higher in developing countries than it is in developed [1]. It has reached 305 per 100,000 live births based on the 2015 Intercensus Population Survey. The newborn mortality rate is 15 per 1,000 live births, based on the Indonesian Health Demographic Survey [2]. Preeclampsia is characterized by the early onset of hypertension, edema, proteinuria, chronic immune activation and endothelial dysfunction, after 20 weeks of gestation [3]. This pregnancy complication is indicated by a systolic/diastolic blood pressure of 140/90 mmHg or higher, proteinuria of more than 300 mg or a protein-creatinine ratio higher than 0.3 in 24-hour urine [4]. In preeclampsia, trophoblasts experience increased oxidative stress on trophoblasts along with increased inflammatory response along with decreased antioxidants [5]. The pathogenesis of preeclampsia stems from cytotrophoblast differentiation, invasion of the superficial uterine cytotrophoblast followed by decreased maternal blood flow to the placenta [6].

Cysteine-dependent aspartate-driven proteases (caspases) are synthesized in cells as zymogens. Apoptosis is associated with Caspase activation [7]. Apoptosis is defined as programmed cell death accompanied by morphological changes [8]. The molecular changes in apoptosis are the release of cytochrome c from the mitochondria and the activation of caspase 3 in the cytosol [9]. Intrinsic apoptosis-mediated permeabilization of the mitochondrial outer membrane leads to apoptosis formation, activation of caspase-9, and effector caspase. Caspase-3, caspase-7 affect the regulation of ROS production during intrinsic cell death [10]. Effector caspases including caspase-3 and -7 which are located in the cytosol, cytoskeleton, and nucleus, during apoptosis can cleave the P57 subunit in mitochondria which damages the electron transport chain and ends with a reorganization of the actin cytoskeleton and membrane blebbing [11].

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Preeclampsia causes a maternal systemic inflammatory response syndrome involving leukocytes and endothelial cells, oxidative stress that damages maternal syncytiotrophoblast cells, and apoptosis [12]. Microdeposition of the syncytiotrophoblast-microvillus membrane occurs in systemic endothelial damage [13]. In addition, apoptosis in trophoblast cells is a clinical manifestation of preeclampsia [14]. A higher inflammatory response indicates an increased release of proinflammatory cytokines such as interleukin-6 (IL-6) [15].

Interleukin-6 (IL-6) was found to be widely expressed in the female reproductive tract as well as in pregnancy tissue, regulating the function of embryo implantation and placental development, immune adaptation to gestational tolerance [16]. Interleukin-6 is involved in chronic inflammation, and autoimmunity [17]. Furthermore, adaptive immunity is stimulated by excessive apoptosis. Monocytes are the largest type of leukocyte and show increased expression of IL-6 in the uterus, decidual cells, and the placenta [18].

Spirulina is a blue-green algae that contains 60%-70% protein, 15%-25% carbohydrates, 6%-13% fat, and 8%-10% fiber [19]. It has a microscopic filamentous cyanobacterium containing C-phycocyanin (CPC) [20]. Moreover, it induces apoptosis by DNA fragmentation and nuclear condensation. C-phycocyanin from spirulina reduces regulation of antiapoptotic proteins and upregulates proapoptotic proteins [21]. It functions as an antioxidant. Furthermore, it inhibits the development of immunomodulators [22,23]. In this study, we investigated the effect of spirulina on apoptosis in trophoblast cells and Caspase 3 expression in the IL-6-induced rat model of preeclampsia.

Material and Methods

This research has been reviewed by the appropriate ethics committee of the Faculty of Medicine, Wijaya Kusuma University, Surabaya (No. 10198/SLE/FK/UWKS/2018). This study was Post Test Only Control Group Design; twenty five rats were divided into 5 groups. These groups are similar to the research conducted by Soekidjo [24]:

Negative control: pregnant rats were given aquadest
P0 (positive control): induced by IL-6 at a dose of 5 mg/day (intravenous [IV]),
P1 (treatment group 1): induced by IL-6 and given by spirulina at a dose of 10 mg/day (oral),
P2 (treatment group 2): induced by IL-6 and administered by spirulina at a dose of 20 mg/day (oral),
P3 (treatment group 3): induced by IL-6 and administered by spirulina at a dose of 40 mg/day (oral),

Two to three month old female rats weighing between 150 and 250 gm were separated from male rats for 2 weeks. Male and female rats were then put together into cages after 2 weeks. A vaginal plug-in female mice was examined after night. Pregnant rats were divided into five groups, each group containing five rats. Mice treated with AIN 93G food from Dyets Inc. and distilled water. The ingredients of AIN 93G are as follows: 40% flour, 20% casein, 13% maltodextrin, 10% sucrose, 7% soybean oil, 5% fiber, 3.5% minerals, 1% vitamins, 0.3% L-cystine, 0.25% choline bitartrate, and 0.0014% tert-butylhydroquinone. Mice were treated at a dose of 5mg/100 g using a wing needle in the tail vein for 3 days. Systolic and diastolic blood pressure was measured with a transducer three times (Kent Scientific CODA) at the Physiology Laboratory, Faculty of Medicine, Universitas Brawijaya. The average value of systolic and diastolic pressure was measured.

Spirulina is injected at a dose of 10 (P1), 20 (P2), and 40 (P3) mg/day. The control group and the treatment group were acclimatized with ketamine 0.5 mg orally after treatment. The rat placenta was placed in an Eppendorf tube containing 10% formalin. Then, the placenta was placed on a glass slide and stained using Bax polyclonal antibody with FITC and Rhodamine staining reagents. Observations were made using a confocal microscope. Expression of apoptotic cells and caspase-3 in placenta using Bax polyclonal antibody staining with FITC and Rhodamine.

Results and Discussion

The results of this study were analyzed using comparative and homogeneity tests. Caspase-3 expression and apoptosis were measured using one-way ANOVA and Post Hoc Fisher's Least Significant Difference comparative test. As presented in Table 1, caspase-3 in the preeclampsia model is represented in P0 which has the highest level compared to other groups. Between treatment groups (P1, P2, P3), the expression of caspase-3 at P3 refers to the lowest level, close to P0.

Table 1.Caspase-3 Expression and Apoptosis

Caspase3	N	Mean	Standard Deviation	P
Negative Control	5	202.77	16.72	.003
P0	5	804.33	19.40	.080
P1	5	726.00	21.29	.001
P2	5	667.63	23.04	.060
P3	5	592.24	19.40	.009
Apoptosis				
Negative Control	5	46.32	6.08	.000
P0	5	62.38	13.28	.090
P1	5	62.96	2.38	.000
P2	5	52.10	12.42	.000
P3	5	44.26	9.41	.018

Apoptotic expression showed a slightly different trend with caspase-3 expression. The lowest level of apoptotic cells in the placenta was at P3 which was given with

spirulina at a dose of 40 mg/day (the highest dose). This indicates that spirulina at a dose of 40 mg/day can restore apoptosis in preeclampsia model rats (44,26) compared to negative controls (46,32). The spirulina treatment group at a dose of 10 mg/day (P1) significantly increased the rate of apoptosis.

Elevated levels of IL-6 have been reported in preeclamptic pregnancies. IL-6 increases endothelial cell permeability in pregnancy pathology by altering the shape and rearrangement of intracellular actin fibers. Furthermore, it reduces prostacyclin synthesis by inhibiting the cyclooxygenase enzyme and increasing the ratio of thromboxane A2 to prostacyclin. IL-6 also stimulates platelet-derived growth factors that cause preeclampsia [25]. In a previous study, the induction of IL-6 in experimental animals led to an increase in the expression of apoptosis. Increased lL-6 production through monocyte activation and Th1 predominance induces inflammation due to dysregulation of the maternal immune system [26]. In addition, it regulates immune and inflammatory responses [27]. Therefore, endothelial cell dysfunction induces preeclampsia by increasing blood plasma levels and impairing the immune system [28]. This is indicated by the average expression of caspase-3 in the positive control group. In the negative control, the expression of apoptosis was lower than the positive control.

This phenomenon is similar to a study[29] entitled Phycocyanin ameliorates trophoblast apoptosis in a mouse model of IL-6-induced preeclampsia. Spirulina's bioactive content is carotenoids, phycocyanin, beta-carotene, zeaxanthin, SOD, polysaccharides, calcium, GLA, Vitamin B-12, Vitamin K1 and K2, it also contains high fiber and protein which are used for health maintenance. Spirulina is useful in preventing and controlling preeclampsia by reducing levels of proinflammatory cytokines [20,22,29]. Spirulina offers a more complete nutritional profile than phycocyanin, which is why there is a hope that it will be better than both. The following are the histopathological results of caspase 3.

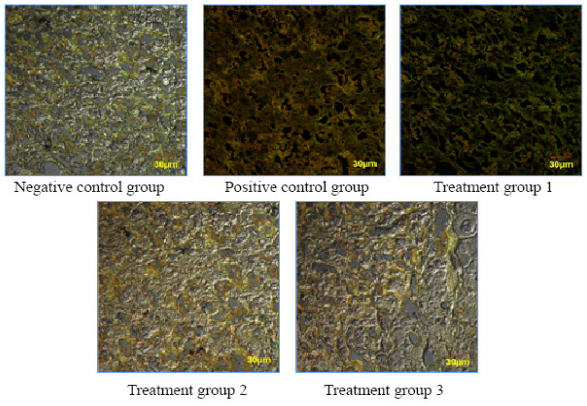

Figure 1. Histopathological results on Caspase-3

After administering spirulina to a pregnant rat model of preeclampsia that had been induced by IL-6, there was a decrease in apoptosis and caspase-3 expression as shown in Figure 1. The test was performed by counting the number of cells containing caspase-3 expression with 400x magnification in 10 fields of view. On examination, it was found that the expression of caspase-3 after administration of spirulina at treatment 3 doses of 40 mg/day/kg BW decreased apoptotic expression more than before the administration of spirulina.

The doses of P1 and P2 were significantly different ($p>0.05$). in the P1 group, the average expression of caspase-3 was 726.00 and apoptosis 62.38. together with P2, the mean expression values of caspase-3 and apoptosis were 667.63 and 52.10, respectively. These results indicate that caspase-3 inhibition is significantly reduced by apoptosis. Spirulina therapy contains CPC, which suppresses caspase 3. Therefore, the apoptotic pathway can also be suppressed [29,30]. We found that spirulina at a dose of 40 mg/day reduced trophoblast cell apoptosis. These results are in aagreement with the theory which states that Spirulina plantesis may help the immune system to fight against infection. This alga also contains gamma-linolenic acid (GLA), and also provides alpha-linolenic acid (ALA), linolenic acid (LA), stearidonic acid (SDA), eicosapentaeonic (EPA), docosahexaenoic acid (DHA), arachidonic acid (A A). The vitamins contained in it are vitamins B1, B2, B3, B6, B9, B12, Vitamin C, Vitamin D, and Vitamin E. 2,3. In addition it is also a source of potassium, calcium, chromium, copper, iron, magnesium, manganese, phosphorus, selenium, sodium, and zinc which are used by the wider community to maintain a healthy body, especially the content of C-phycocyanin which is known as C-PC, a substance with potent cancer chemopreventive activity [20] to prevent and control the occurrence of preeclampsia, by decreasing levels of pro-inflammatory cytokines [29].

Conclusion

The administration of spirulina 40 mg/day orally was effective in repairing trophoblasts and reducing the expression of apoptosis and caspase 3 in an IL-6-induced rat model of preeclampsia.

Suggestion

Further studies should be conducted with a longer treatment period of spirulina administration to elucidate the significant effect of spirulina on the reduction of apoptosis. Additionally, spirulina's dose should be considered to determine its toxicity.

Confession

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