Effect of phycocyanin on expression of malondialdehyde and interferon-γ placental trophoblast cells models of preeclampsia rats

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Abstract
Preeclampsia is a "disease of theory" because of the cause and pathophysiology of preeclampsia is still uncertain. In preeclampsia invasion of trophoblast cells does not occur completely, the spiral arteries not undergo remodeling so it still has a component of muscle and elastic tissue, in addition to endothelial dysfunction characterized by reduced relaxation factor and increased contraction factor resulting in ischemia of the placenta by The end result of hypoxia, hypoxia is the condition that causes the production of free radicals in large quantities. Free radicals can damage all the cellular biochemical components of lipids by taking lipid electrons in a cell membrane called lipid peroxidation. Lipid peroxidation process produces several products including Malondialdehyde (MDA) also mediate proinflammatory cytokines such expenditure Interferon Gamma (IFN-γ). In this study, researchers took the form of placental tissue paraffin blocks which have been made, on each of the paraffin block containing a number of 6 groups of placental tissue (30 blocks of paraffin). The method used is double staining with the reading of 3 field trophoblast cells with 200x magnification. On examination Malondialdehyde done by TBARS and analyzed by immunoflourence by staining rhodamine and DAPI, while on inspection of IFN-γ of the preparations that has been staining with antibodies PE anti-rat IFN-γ, and DAPI were analyzed using immunoflorescence, then interpreted using software immuno flow. Provision of Phycocyanin in this study proved to reduce levels of MDA and Interferon-γ. Phycocyanin dose 40 ng / 100 grams BB which has an effective dose decreases MDA and Interferon-γ levels, Because of phycocyanin has a role as an anti-inflammatory, antioxidant and immunomodulatory. In giving of Phycocyanin which in large doses can trigger an increase in proinflammatory cytokines. Further research is needed on the protective effect of Phycocyanin on human placental tissue.

Keywords: Preeclampsia; Phycocianin; Malondialdehyde; IFN-γ

1. Introduction
Preeclampsia is a worldwide cause of morbidity and mortality characterized by hypertension and excessive urinary protein excretion after 20 weeks' gestation [23,25,29]. Preeclampsia is called "disease of theory" because of the cause and pathophysiology of preeclampsia is still uncertain [16,22]. In normal pregnancy, trophoblast proliferation invades decidua and myometrium occurs in 2 stages, first-stage invasion begins with endovascular trophoblast cells invading the spiral arteries [11,12,13], while second-stage invasion of trophoblast cells invades the spiral artery to the myometrium [13]. Invasion of trophoblast cells causes spiral artery changes such as damage to the muscle layer, elastic layer, and nerve tissue found in the spiral artery wall and endothelial cell replacement with cytrophoblast cells. Remodeling of the spiral arteries results in spiral arteries having thin, limp, larger-diameter walls that can adjust for increased blood flow requirements during pregnancy and developing fetuses [25,6]. In pre-eclampsia the invasion of...
lipids by taking lipid electrons in a cell

given Phycocyanin at doses 10, 20, 40 and 80 ng / 100 gr BB done by using ANOVA. Normality test using Saphiro-Wilk test, said to be fulfilled if p-value of calculation result is bigger than α = 0.05 using SPSS software aid with result as follows:

2. Material and methods
This research is experimental research with posttest control design method. In this study the researchers took the parameters of an existing study in the form of placental tissue that has been made paraffin blocks, in each paraffin block containing placental tissue, previous researchers have given treatment in the form of IL-6 induction with dose 5ng / grams BB rat for 5 Day through the vein of the tail in the bunting rats aged 10 days pregnant and giving phycocyanin with a dose of 10, 20, 40.80 ng. Animal experiments before induction of IL-6 induction of 30 white mice was performed by synchronizing the estrous cycle with Leeboth, Pheromone, Whitten effect aimed at obtaining homogeneous / pregnant-aged bunting rats. This research was conducted in anatomical pathology laboratory of UB faculty of medicine, biology laboratory and biomedical laboratory of Universitas Brawijaya Malang. In this study using a paraffin block sample containing placental tissue of 6 groups (30 paraffin blocks) consisting of a negative control paraffin block (N) containing a network of experimental animals without IL-6 induced and without phycocyanin, paraffin blocks Positive control (K) containing IL-6 IL-6 induced dose tissue 5 d / day for 5 days, paraffin block Treatment 1 (P1) containing IL-6 induced experimental tissue dose 5 ng / day + phycocyanin dose 10 ng during 5 days, paraffin blocks Treatment 2 (P2) containing IL-6 IL-6 induced dose tissue dose 5 ng / day + phycocyanin dose 20 ng for 5 days, paraffin Treatment Block 3 (P3) containing IL-6 doses 5 ng / day + phycocyanin dose 40 ng for 5 days, paraffin Treatment Block 4 (P4) containing tissue content of IL-6 induced 5 ng / day + phycocyanin dose 80 ng for 5 days. Inclusion criteria paraffin blocks containing placental tissue and in good condition, Exclusion criteria Paraffin blocks are damaged and not possible to do research. The method used is double staining with the reading of 3 field trophoblast cells with 200x magnification. Malondialdehyde examination was performed with TBARS and analyzed with immunofluorencence with rhodamine stain and dapi, whereas on IFN-γ examination of preparations that had been stained with IFN-PE anti-rat PE antibody, and was analyzed using immunofluorescence, then interpreted using immunoflow software.

3. Results
Testing of MDA and Interferon-γ trophoblast cell levels on Saved Biological Material (BBT) containing placental tissue consisted of 2 control groups (positive control and negative control) and 4 treatment groups given Phycocyanin at doses of 10, 20, 40 and 80 ng / 100 gr BB done by using ANOVA. Normality test using Saphiro-Wilk test, said to be fulfilled if p-value of calculation result is bigger than α = 0.05 using SPSS software aid with result as follows:
Table 1 Normality test

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>P-value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA</td>
<td>0.959</td>
<td>0.722</td>
<td>Normal</td>
</tr>
<tr>
<td>Interferon-γ</td>
<td>0.983</td>
<td>0.882</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Homogeneity test was performed using Levene test. Homogeneity is said to be fulfilled if p-value of calculation is bigger than $\alpha = 0.05$, here is homogeneity test result:

Table 2 Test Homogeneity Variety

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>P-value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA</td>
<td>1.561</td>
<td>0.198</td>
<td>Homogeneous</td>
</tr>
<tr>
<td>Interferon-γ</td>
<td>1.498</td>
<td>0.243</td>
<td>Homogeneous</td>
</tr>
</tbody>
</table>

In this study, there was decreased MDA levels after Phycocyanin administration, although it could not achieve MDA levels as a negative control group.

Table 3 MDA expression

<table>
<thead>
<tr>
<th>Paraffin blocks containing rat placenta tissue with IL-6 5ng / 100 grams BB induction for 5 days</th>
<th>Mean ± SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Controls</td>
<td>422.15 ± 19.10</td>
<td>b</td>
</tr>
<tr>
<td>Positive Control</td>
<td>650.80 ± 22.99</td>
<td>d</td>
</tr>
<tr>
<td>Dose of Phycocyanin 10 / 100 grams BB</td>
<td>583.87 ± 11.75</td>
<td>f</td>
</tr>
<tr>
<td>Dose Phycocyanin 20 / 100 grams BB</td>
<td>553.40 ± 24.12</td>
<td>c</td>
</tr>
<tr>
<td>Dose of Phycocyanin 40 / 100 grams BB</td>
<td>454.41 ± 18.75</td>
<td>a</td>
</tr>
<tr>
<td>Dose Phycocyanin 80 / 100 grams BB</td>
<td>585.53 ± 6.44</td>
<td>e</td>
</tr>
</tbody>
</table>

Based on multiple comparison test with LSD test, different MDA levels were found in negative control group, positive control and Phycocyanin administration with different dose. In the treatment group dose of Phycocyanin 10 ng / 100 grams BB obtained MDA level (583.87 ± 11.75); Group of Phycocyanin 20ng / 100 grams BB dose treatment obtained MDA level (553.40 ± 24.12); Phycocyanin 40ng / 100 grams BB dose treatment group obtained MDA level (454.41 ± 18.75) and treatment group dose of Phycocyanin 80ng / 100 grams BB obtained MDA level (662.21 ± 17.44).
Figure 1 Results of MDA Immunofluorecence Examination on rat trophoblast model of preeclampsia study

Description: A: Negative control group; B: Positive control group; C: Treatment group with Phycocyanin dose 10 ng / BB; D: Treatment group with Phycocyanin dose 20 ng / BB; E: Treatment group with Phycocyanin dose 40 ng / BB; And F: Treatment group with dose of Phycocyanin 80 ng / BB
Figure 2 Diagrams shows comparison of mean MDA levels in negative control group, positive control and Phycocyanin group

Description: The administration of this Phycocyanin may decrease mean MDA levels after induction with IL-6 in pregnant mice of preeclampsia model. In all doses of Phycocyanin there was a decrease in mean MDA levels post-induced IL-6 compared to the positive control group. In the dosage group Phycocyanin 40ng / 100 grams BB showed the lowest values of MDA levels compared with other doses of Phycocyanin.

In this study, biologically stored material (BBT) in the form of paraffin blocks and containing rat placental tissue with IL-6 induction of 5ng / 100 grasmss rat for 5 consecutive days in this study also found a significant decrease in Interferon-pada in positive group (656.56 ± 46.56) compared with the treatment group. The study found Interferon-penurunan decrease after Phycocyanin administration, although it could not reach Interferon-γ as a negative control group.

Table 4 Interferon-γ expression

<table>
<thead>
<tr>
<th>Paraffin blocks containing rat placenta tissue with IL-6 5ng / 100 grams BB induction for 5 days</th>
<th>Mean ± SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Controls</td>
<td>460.81 ± 28.24</td>
<td>c</td>
</tr>
<tr>
<td>Positive Control</td>
<td>656.56 ± 46.56</td>
<td>a</td>
</tr>
<tr>
<td>Dose of Phycocyanin 10 / 100 grams BB</td>
<td>566.69 ± 11.38</td>
<td>b</td>
</tr>
<tr>
<td>Dose Phycocyanin 20 / 100 grams BB</td>
<td>534.18 ± 17.5</td>
<td>e</td>
</tr>
<tr>
<td>Dose of Phycocyanin 40 / 100 grams BB</td>
<td>420.31 ± 10.27</td>
<td>d</td>
</tr>
<tr>
<td>Dose Phycocyanin 80 / 100 grams BB</td>
<td>568.77 ± 1.18</td>
<td>f</td>
</tr>
</tbody>
</table>

Based on multiple comparison test with LSD test, interferon-diperoleh obtained significantly different in negative control group, positive control and giving of Phycocyanin with different dose. In the treatment group dose of Phycocyanin 10 ng / 100 grams BB Interferon-γ obtained (566.69 ± 11.38); Phycocyanin 20ng / 100 grams BB dose treatment group obtained Interferon-γ (534.18 ± 17.5); Phycocyanin 40ng / 100 grams BB dose treatment group obtained Interferon-γ amount (420.31 ± 10.27) and treatment group dose of Phycocyanin 80ng / 100 grams BB obtained Interferon-γ (568.77 ± 1.18).
Figure 3 Results of Intermittent Interferon-Im Immunofluorescence Examination on rat trophoblast model of preeclampsia study

Description: A: Negative control group; B: Positive control group; C: Treatment group with Phycocyanin dose 10 ng / BB; D: Treatment group with Phycocyanin dose 20ng / BB; E: Treatment group with Phycocyanin dose 40 ng / BB; And F: Treatment group with dose of Phycocyanin 80ng / BB
Figure 4：Diagrams shows the average comparison of Interferon-γ in the negative, positive and Phycocyanin groups.

Description: Giving this Phycocyanin can decrease mean Interferon-γ post induction with IL-6 in pregnant mouse model of preeclampsia. In all doses of Phycocyanin there was a decrease in mean Interferon-γ post induction of IL-6 compared to the positive control group. In the dose group Phycocyanin 40ng / 100grams BB showed the lowest values of Interferon-γ mean compared with other doses of Phycocyanin.

Treatment with Phycocyanin at different doses showed a significant decrease in interferon-γ, wherein Interferon-γ was lowest on the use of a Phycocyanin dose of 40ng / 100grams BB rat (420.31 ± 10.27). For dosing of Phycocyanin with doses of 10 and 20 / 100grams BB mice did not show significant mean Interferon-γ difference. In the treatment group with a dose of 40ng / 100 grams of rat BB, the mean interferon-γ was different with the positive control group. In the treatment group with the dose of 80 ng / 100 grams BB obtained mean Interferon-γ which is different from the positive control group. In the treatment group with Phycocyanin at different doses showed a decrease in Interferon-γ.

4. Discussion

Provision of Phycocyanin in this study proved to reduce levels of MDA and Interferon-γ. Phycocyanin dose 40 ng / 100grams BB which has an effective dose decreases MDA and Interferon-γ levels. Phycocyanin dose 40 ng / 100grams BB which has an effective dose lowers levels of MDA and Interferon-γ. Because Phycocyanin has a role as anti-inflammatory, antioxidant and immunomodulator. In giving of Phycocyanin dose 80ng / 100grams BB got Interferon-γ. Compared to a dose of Phycocyanin 40 ng / 100 grams BB associated with a dose of Phycocyanin, which in large doses may trigger an increase in proinflammatory cytokines.

5. Conclusion

Further research is needed on the protective effect of Phycocyanin on human placental tissue by tissue culture so that Phycocyanin is actually shown to protect preeclampsia and can be given clinically.

Compliance with ethical standards

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Disclosure of conflict of interest

We warrant that the article is the Authors' original work and ensure no conflicts of interest to declare. We certify that the submission is not under review at any other publication.
References


