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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 immunoflourence, then interpreted using software imunoflow. Provision of Phycocyanin in this study proved to reduce levels of MDA and Interferon-γ. Phycocyanin dose 40 ng / 100 gramss 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 cytotrophoblast 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

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trophoblast cells does not occur completely, the spiral arteries present in the myometrium do not undergo remodeling so that they have both muscle and elastic tissue, but endothelial dysfunction is characterized by a decrease in the substance of endothelium Derived Relaxing Factors EDRFs) and increased contraction factors or so-called Endothelium Derived Contracting Factors (EDCFs) [17,21,32]. The decrease in relaxation factor followed by increased contraction factor resulted in placental ischemia with the end result of hypoxia, the hypoxic condition causing the generation of free radicals in large quantities [1,3,15]. Free radicals that produce more than antioxidants cause a condition called oxidative stress. Free radicals can damage all the cellular biochemical components of lipids by taking lipid electrons in a cell membrane called lipid peroxidation [9,27,28]. The lipid peroxidation process produces several products including Malondialdehyde (MDA). Several studies have been conducted on lipid peroxidation in preeclampsia, achieving significantly higher MDA levels in patients with preeclampsia than with normal pregnancies. MDA is a biomarker that shows free radicals and is used to assess oxidative stress [29,34]. Oxidative stress in addition to damaging the biochemical components which one of them is lipid also mediate the release of pro-inflammatory cytokines such as Interferon Gamma (IFN-γ), TNF-α, IL-6. Inadequate therapy to prevent preeclampsia is not present [35,37]. Some studies related to Phycocyanin have been shown to have immunoregulatory effects by stimulating various immune functions such as cytokine production, chemokines, anti-inflammatory mediators, NK cell activity, production of B cell antibodies and T cell proliferation [30,31,33]. Research on the administration of spirulina with active ingredient Phycocyanin is able to control the occurrence of preeclampsia, through decreased levels of pro-inflammatory cytokines (Th1) among which are IL-6, TGF-β, and IFN γ [4,7,8,10]. In addition to having immunoregulatory effects Phycocyanin also has anti-inflammatory and anti-oxidant effects, where its antioxidant effects have been tested in vitro to inhibit lipid peroxidation initiated by free radicals [14,19,26]. Based on the description above, shows that phycocyanin is thought to be able to provide protective effects on preeclampsia because it has immunoregulation, anti-inflammatory and anti-oxidant effects, where the effects of immunoregulation and anti-inflammatory can reduce levels of pro-inflammatory cytokines, in addition the anti-oxidant effect also can inhibit lipid peroxidation by Therefore researchers interested in doing research on the effects of phycocyanin to levels of Malondialdehyde and interferon gamma trophoblast cells in the mouse model of preeclampsia [38,40].

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 dose 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 immunoflourence 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 immunofluoresence, 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 $\alpha = 0,05$ using SPSS software aid with result as follows:

Table 1 Normality test

Variable	Coefficient	P-value	Description
MDA	0.959	0.722	Normal
Interferon-γ	0.983	0.882	Normal

ity test was performed using Levene test. Homogeneity is said to be fulfilled if p-value of calculation is bigger than α = 0,05, here is homogenity test result:

Table 2 Test Homogeneity Variety

Variable	Coefficient	P-value	Description
MDA	1.561	0.198	Homogeneous
Interferon-γ	1.498	0.243	Homogeneous

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

Paraffin blocks containing rat placenta tissue with IL-6 5ng / 100 grams BB induction for 5 days	**Mean ± SD**		**p-value**
Negative Controls	422.15 ± 19.10	b	0.000
Positive Control	650.80 ± 22.99	d	
Dose of Phycocyanin 10 / 100 gramss BB	583.87 ± 11.75	f	
Dose Phycocyanin 20 / 100 gramss BB	553.40 ± 24.12	c	
Dose of Phycocyanin 40 / 100 gramss BB	454.41± 18.75	a	
Dose Phycocyanin 80 / 100 gramss BB	585.53±6.44	e	

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 / 100grams BB obtained MDA level (583.87 ± 11.75); Group of Phycocyanin 20ng / 100grams BB dose treatment obtained MDA level (553.40 ± 24.12); Phycocyanin 40ng / 100grams BB dose treatment group obtained MDA level (454.41 ± 18.75) and treatment group dose of Phycocyanin 80ng / 100grams BB obtained MDA level (662.21 ± 17.44).

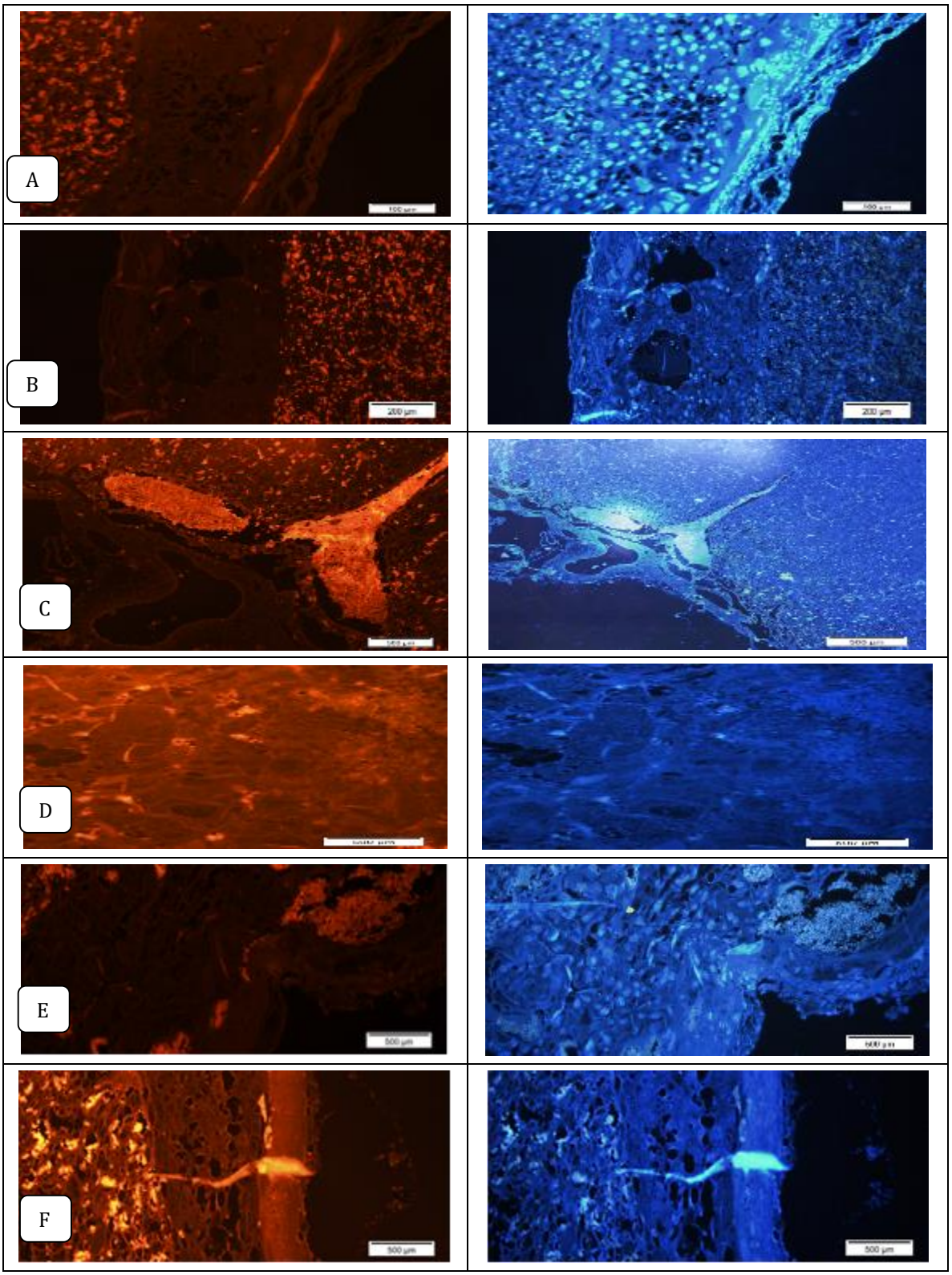

Figure 1 Results of MDA Immunofluorence Examination on rat trophoblast model of preeclampsia study

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

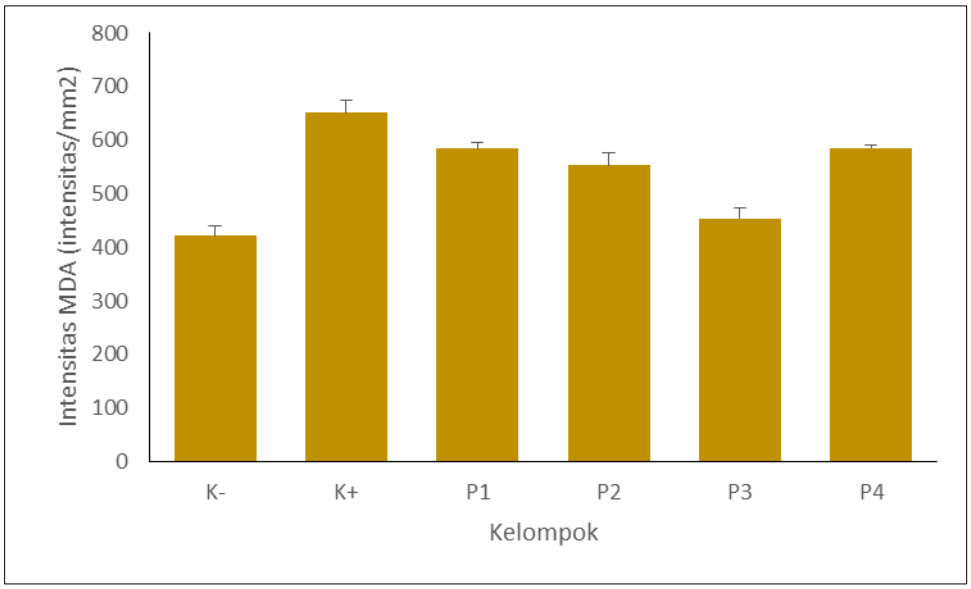

Figure 2 Diagrams shows comparison of mean MDA levels in negative control group, positive control and Phycocyanin group

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 / 100grams 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 5 ng / 100 gramss 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

Paraffin blocks containing rat placenta tissue with IL-6 5ng / 100 grams BB induction for 5 days	**Mean ± SD**		**p-value**
Negative Controls	460.81 ± 28,24	c	0.000
Positive Control	656.56 ± 46,56	a	
Dose of Phycocyanin 10 / 100grams BB	566.69 ± 11.38	b	
Dose Phycocyanin 20 / 100grams BB	534.18 ± 17.5	e	
Dose of Phycocyanin 40 / 100grams BB	420.31 ± 10.27	d	
Dose Phycocyanin 80 / 100grams BB	568.77±1.18	f	

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 / 100grams BB Interferon-γ obtained (566.69 ± 11.38); Phycocyanin 20ng / 100grams BB dose treatment group obtained Interferon-γ (534.18 ± 17.5); Phycocyanin 40ng / 100grams BB dose treatment group obtained Interferon-γ amount (420.31 ± 10.27) and treatment group dose of Phycocyanin 80ng / 100grams BB obtained Interferon-γ (568.77 ± 1.18).

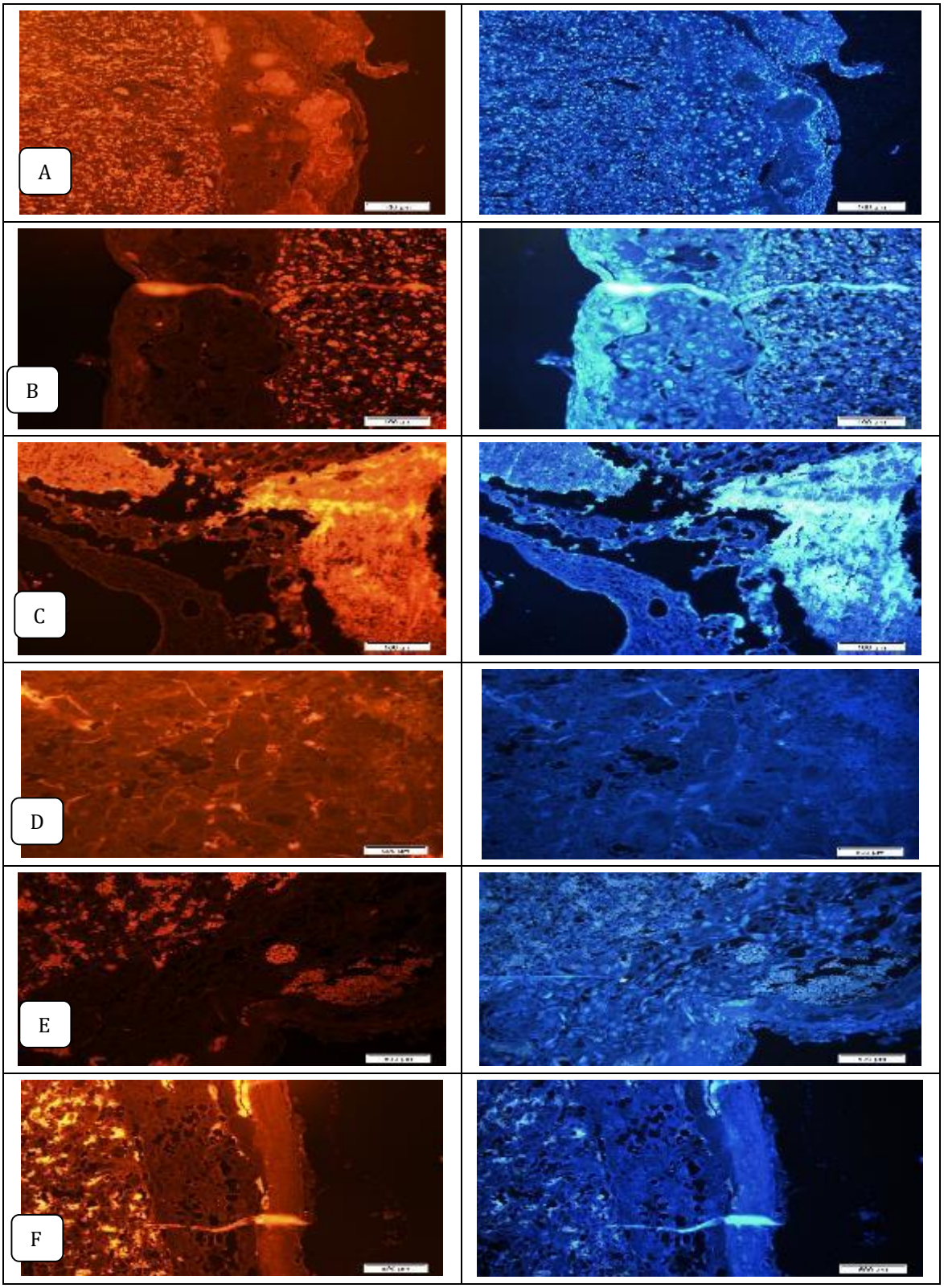

Figure 3 Results of Intermittent Interferon-Im Immunofluorence Examination on rat trophoblast model of preeclampsia study

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

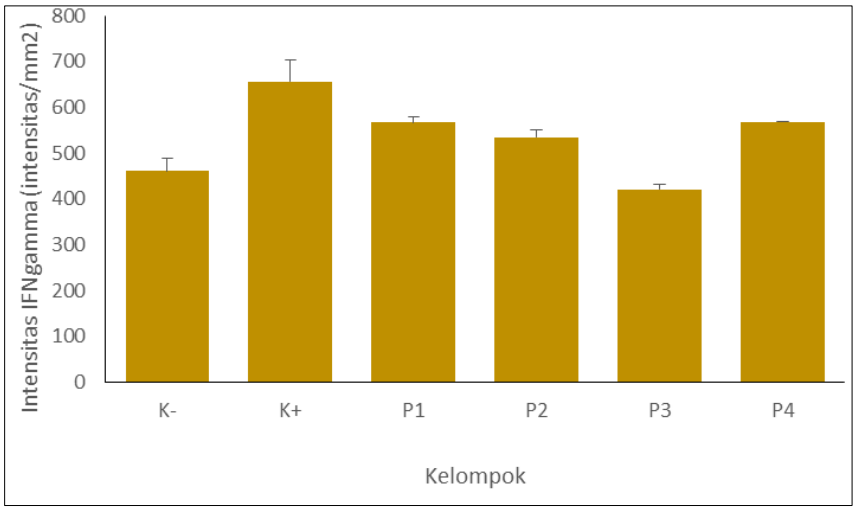

Figure 4 Diagrams shows the average comparison of Interferon-γ in the negative, positive and Phycocyanin groups

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-r mean compared with other doses of Phycocyanin.

Treatment with Phycocyanin at different doses showed a significant decrease in interferon-„ 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-r difference. In the treatment group with a dose of 40ng / 100 gramss of rat BB, the mean interferon-r was different with the positive control group. In the treatment group with the dose of 80 ng / 100 gramss BB mice 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

[1] Abbas AK, Lichtman AH, Pillai S. Cellular and Molecular Immunology. 7th edn. Philadelphia, PA: Saunders/Elsevier; 2012.

[2] Allaire AD, Ballenger KA, Wells SR, McMahon MJ, Lessey BA. Placentall apoptosis in preeclampsia. Am J Obstet Gynecol. 2000;96:271-6.

[3] Ana C. Palel, Frank T. Spradley, Junie P. Warrington, Eric M. George, and Joey P. Granger. 2013. Pathophysysiology of Hypertension in Preeclampsia: Lesson in Integrative Physiology. National Institute of Health. 208 (3): 224-233.

[4] Andrea T.B, Belay A, Keen C.L. 2008. Spirulina and Immunity. In :Spirulina In Human Nutirion and Health. Taylor Francis Group, United Stated America. 117 – 94.

[5] Arbogast BW, Taylor RN. 1996 . Medical Inteligence Unit. Molecular Mechanism of Preeclampsia. Austin: R.G. Landes company:1–54.

[6] Assche FA, Pijnenborg R. 1999. The Pathology of Preeclampsia/Eclampsia. In: Killick SR, et al., editors. Gynecology forum 4(1): 10–12.

[7] Belay A. 2012. The Potential Application of Spirulina (Arthrospira) as a Nutritional and Therapeutic Supplement in Health Management. The Journal of the American Nutraceutical Association 5(2).

[8] Borchers AT., Belay A., Keen., Gershwin ME. 2008. Spirulina and Immunity. In :Spirulina in Human Nutrition and Health.Eds, ME Gershwin, Belay A. Taylor & Francis, p 177 – 194.

[9] Bravo J., Heath J.K. Receptor recognition by gp130 cytokines. EMBO J. 2000;19:2399–2411.

[10] CollaLM., Furlong EB., Costa JA. 2007. Antioxidant Properties of Spirulina (Arthospira) platensisCultivated Under Different Temperatures and Nitrogen Regimes. Brazilian Archives Of Biology And Technology Journal. Vol.50, n. 1 : pp.161-167

[11] Cross J. 2006. Trofoblast Fate Cell Specification. In Biology and Pathology Trofoblast. Cambridge University Press. United of Kingdom. 3-15.

[12] Cunningham, F Gary. etal.,l. 2010. Obstetri Williams23rd ed. USA : The McGraw-Hill Companies, Inc.

[13] Cunningham, F.G, Leveno, K.J. 2014. Implatation, embryogenesis, and placental development. In Williams Obstetric. 24th edition. Editors Cunningham. United State Of America, McGraww Hills Medical, 14 -174.

[14] Davey DA. Hypertension Disorders of Pregnancy. 1997. In: Whitfield CR, editor. Dewhurst's text book of obstetric and gynecology for post graduates. 5th Ed. USA: Black Well Science:175–213.

[15] Fonseca BM., Silva GC., Teixeira NA. 2012. The Rat as an Animal Model for Placental Development :A Reappraisal of The Post Implantation Period. Reproductive Biology 12 (2): 97 – 113.

[16] Gadonski G., LaMArca BD., Sullivan E. 2006. Hypertension Produced By Reductions In Uterine Perfusion In The Pregnant Rat : Role Of Interleukin 6. Hypertension American Heart Association 48: 711 – 716.

[17] Gil Mor and Vikki M Abrahams. 2004. Potential role of macrophages as immunoregulators of pregnancy.ReprodBiolEndocrinol. doi: 10.1186/1477-7827-1-119 1: 119.

[18] Hammid HY., Zakaria Ab. 2013. Review :Reproductive Characteristic of the Female Laboratory rat.Academic Journal12 (19): 2510 – 14.

[19] Hedele Champion, Barbara A. Innes, Stephen C. Robson, Gendie E. Lash, and Judith N. Bulmer. 2012. Effects of interleukin-6 on extravillous trophoblast invasion in early human pregnancy. Molecular Human Reproduction 18: 391–400

[20] Heinrich PC, Berhmann I, Newen GM. 2008. Interleukin-6 Type Cytokine SignallingThroughtThe GP130/JAK/STAT Pathway.Biochemistry Journal334: 297 – 314.

[21] Hoebe K, Janssen E, Beutler B .2004. The interface between innate and acquired immunity. Nat Immunol 10:971–974

[22] Jones S.A. 2005. Directing transition from innate to acquired immunity: defining a role for IL-6. J. Immunol175:3463–3468.

[23] Kathleen A.P, Jessica M.S, Daniel.L.J, Laura.C.S, Danny.J.S. 2012. Preeclampsia: Multiple Approaches for a Multifactorial Disease. Disease Models & Mechanisms. 5: 9-18

[24] Kamimura D, Ishihara K, Hirano T.2003. IL-6 signal transduction and its physiological roles: the signal orchestration model. Rev PhysiolBiochemPharmacol149:1–38.

[25] Keman K, Presetyorini N, Langgar M J. 2009. Perbandinganekspresi p53, Bcl-2, danIndeks Apoptosis TrofoblaspadaPreeklampsia/EklampsiadanKehamilan Normal. MajalahObstetridanGinekologi Indonesia,33(3): 151-159

[26] Kumar N., Kumar P., Sighn P. 2010. Immunomodulatory effect of dietary Spirulinaplatensis in in rats. Research Journal of Pharmaceutical,Biological and Chemical Sciences4(1): 877 – 98.

[27] Leber A, Teles A, Zenclussen C. 2010. Regulatory T Cells and Their Role in Pregnancy. American Journal of Reproductive Immunology63: 445 - 59.

[28] Loockwood CJ, Yen CF, Basar. 2008. Preeclampsia Related Inflammatory Cytokines Regulate Interleukin 6 Expression in Human Decidual Cells. The American Journal of Pathology172: 1571 – 79.

[29] Mark F., McCarty. 2011. A Potential Role for Spirulina in the Prevention and Management of Preeclampsia. NutriGuard Research, Inc., 1051 Hermes Ave., Encinitas, CA 92024.

[30] Mor G, Abrahams VM. 2003. Potential Role of Macgrophage as Immunoregulators of Pregnancy. Reproductive Biology and Endocrinology (I), 1119 – 27.

[31] Mor G, Abrahmas VM. 2006. Tropholast Cells As Immune Regulators. In : Immunology of Preganncy. Editor Gil Mor. United State of America, Landes Springer Science. 215 -44.

[32] Mor G, Cardenas I. 2010. The Immune System in Pregnancy : A unique Complexity. American Journal of Reproductive Immunology63: 425 – 33.

[33] Orshal JM., Khalil RA. 2004. Reduced Endotthelial NO-cGMP Mediated Vascular Relaxation and Hypertension in IL-6 Infused Preganant Rats. Hypertension Journal American Heart Association43: 434 – 444.

[34] Pinenborg R., Vercrusyye C., Carter AM. 2011. Trofoblast Invasion and Uterine Artery Remodeling.In : The Placenta and Human Develomental Programsing. Cambridge University Press. United of Kingdom. 92 – 98.

[35] Pinheiro B. Melina, Filho Martins A. Olindo, Mota L. Paula Ana, 2013. Severe preeclampsia goes along with a cytokine network disturbance towards a systemic inflammatory state, Cytokine 62; 165-173.

[36] Qunader SH., Islam SU.,Saifullah A., Majumder., Hannan J. 2013. In vivo Studies of The Anti Inflamstion Effect of SpirulinaPlantesis. The Pharma Journal2(4): 70 – 80.

[37] Sasmito DS, Ulfiati A., Wardhana A., Nugraheni F., Prdiptasari NF., Zulaifa., et al., 2015. Endoglin Expression and The Level of TGFβ are Increased in The Placental Tissue and Correlated with Low Fetal Weigth in Malaria Infected Mice.The Journal Of Tropical Life Science5: 8 -13.

[38] Scheller J., Chalaris., Arras., John SR. 2011. The Pro and Anti Inflamatory Properties of The Cytokine Interleukin 6.BiochimicaetBiophysicaActa 1813: 876 – 88.

[39] Xie F. 2010. Thesis : Infection and Immunity in Pregnancy and Preeclampsia. The University of Colombia (Vancoucer).

[40] Xiao J.P, Yin Y.X, Gao Y.F, Lau S., Shen F, Zhao M, Chen Q. 2012. The increased maternal serum levels of IL-6 are associated with the severity and onset of preeclampsia. Elsevier Cytokine60 : 856–860.