Effect of Curcumin on sFlt-1 and PlGF Concentration in Preeclampsia Induced HUVEC Cell Line

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ABSTRACT

Preeclampsia is a multisystem disorder characterized by hypertension and proteinuria. Elevated soluble fms-like tyrosine kinase 1 (sFlt-1) and reduced placental growth factor (PlGF) levels are documented in preeclampsia patients. Oxidative stress is known to play a role in the etiopathogenesis of preeclampsia. Hence, antioxidants as physiological protective agents can be used in the treating preeclampsia. In this study, we assessed the antioxidant properties of curcumin in HUVEC cell line cultured in serums collected from normal and preeclamptic pregnant women. The levels of sFlt-1 and PlGF were measured with ELISA. We found that sFlt-1 level and sFlt-1/PlGF ratio decreased significantly (p<0.05) while PlGF level increased significantly (p<0.05) after the curcumin administration with concentrations ranged from 0.977–250 µg/mL. The levels of sFlt-1, PlGF, and sFlt-1/PlGF ratio in preeclampsia-induced cell were equal to those in normal cell after the addition of 7.813 µg/mL curcumin after 24 h incubation. Similar results were obtained for samples with 48 h incubation, in exception with PlGF level in preeclampsia-induced cell that required 31.250 µg/mL curcumin to be equal to that in normal cell line. In conclusion, curcumin to be used in treating preeclampsia, by restoring the balance between proangiogenic factor (PlGF) and antiangiogenic factor (sFlt-1). However, further studies are warranted to elucidate this potency.

INTRODUCTION

Preeclampsia is a multisystem disorder characterized by hypertension and proteinuria after 20 weeks of gestation, and affects 2 to 5% of pregnancies worldwide.[1–3] Preeclampsia is associated with high risks of iatrogenic preterm delivery, intrauterine growth restriction, placental abruption, and perinatal mortality, along with maternal morbidity and mortality.[4,5] The underlying mechanism of preeclampsia remains unclear; however, it is suggested to be due to placental malperfusion from the abnormal remodeling of maternal spiral arteries.[6,7] Elevated circulating maternal serum levels of soluble fms-like tyrosine kinase 1 (sFlt-1), and reduced placental growth factor (PlGF) levels, are documented in preeclampsia onset.[8,9] sFlt-1, an antagonist of PlGF and vascular endothelial growth factor causes vasoconstriction and endothelial damage that may lead to fetal growth restriction and preeclampsia.[10–12] The ratio of sFlt-1 and PlGF is associated with an increased risk of preeclampsia which has been approved as a better predictor of risk than either biomarker alone.[9,13–16]

In preeclampsia, reduced perfusion due to aberrant placentation and shallow trophoblast invasion promotes placental oxidative stress,[17] leading to intravascular inflammatory response and endothelial dysfunction. Many investigations have evaluated antioxidant capacity in the maternal circulation. Although there are mixed results, antioxidant capacity is suggested to decrease in the maternal circulation.[18] Hence, the treatment of antioxidants can be used in preeclampsia. Vitamins C and E, nacetylcysteine, L-arginine, and resveratrol had been

used in treatment trial studies, but their effectiveness is conflicting in various studies.[19] Exploration of other alternative antioxidants should be considered, especially that of natural products.

Recent studies have been focusing on natural antioxidants from plants due to the presence of phenolic constituents in which they are biodegradable and non-toxic products.[20,21] Curcumin (diferuloylmethane) is a naturally occurring yellow pigment isolated from ground rhizomes of the plant *Curcuma longa* L. (Zingiberaceae). Although molecular mechanisms of action of curcumin are not fully understood, it has been reported to exhibit potent anti-inflammatory, anti-tumor, and hypolipidemic properties.[22,23] In addition, the potential of curcumin in treating preeclampsia have been investigated with different biomarkers.[24,25] In this study, we evaluated the effect of curcumin on sFlt-1 and PlGF levels in preeclampsia-induced HUVEC cell line.

MATERIALS AND METHOD

Study design

This *in vitro* study adopted a post-test only control group design. Human Umbilical Vein Endothelial Cell (HUVEC) ATCC CRL 1730 (purchased from American Type Collection Culture) was used and induced with normal and preeclamptic sera that were collected from the normal pregnant women and preeclamptic pregnancy, respectively. Curcumin, purchased from Nanjing Zelang Medical Technology Cina, with a variety of concentrations, was administered to each serum group to investigate its

effect on the level of sFlt-1 and PlGF as well as sFlt-1/PlGF ratio.

HUVEC cell culture preparation

HUVEC cell line was added into a tissue culture flask (25 cm^2) containing RPMI 1640 with 20% (v/v) fetal bovine serum (FBS) qualified, 10% endothelial supplement, and antibiotic-antimycotic (1% penicillin G-streptomycin *solution stabilized* and 1% fungizone amphotericin B) and 1% gentamycin. Cells were incubated at 37°C, 5% CO_2 for 7 days to reach 70-80% confluence. The culture medium was replaced for each 2-3 days.[26]

Exposure of HUVEC cells with healthy and preeclamptic sera

Healthy and preeclamptic sera were obtained from patiens in Dr. Hasan Sadikin Hospital, Bandung. As much as 20 cc blood were collected from each 5 healthy pregnant patients and 5 preeclamptic patients to obtain 10 cc healthy serum and 10 cc preeclamptic serum, respectively. The selection criteria followed that of the published literature.[27] The HUVEC cells were then incubated using the same previous procedure, where 20% FBS was replaced with the obtained healthy and preeclamptic sera.[26,28,29]

Measurement of sFlt-1 and PlGF levels

HUVEC cells (6×10^5 cell/ml) that had been incubated with either preeclamptic or normal sera, were applied into 96-wells plate, then incubated at 37 °C 5% CO_2 (v/v). Cells were further washed repeatedly (3-4 times) with PBS at 37°C to remove the remaining medium and cells. Curcumin was aliquoted in various concentrations (0; 0.977; 1.953; 3.906; 7.813; 15.625; 31.25; 62.5; 125; 250 µg/mL), distributed in each well, and incubated for 24 and 48 h at 37°C, 5% CO_2 (v/v). The levels of sFlt-1 and PlGF were quantitatively measured with enzyme-linked immunosorbent assay (ELISA). Absorbance was read using a Thermo Model 680 XR microplate reader (Bio-Rad, California, USA) at 450 nm wavelength.[26,29]

Data analysis

Data were statistically analyzed with a two-way analysis of variance (ANOVA) and continued with Tukey pos hoc using SPSS 21 (IBM Corp, Armonk, NY, USA). The analysis aimed to test the significance of the effect of curcumin in a varied concentration on sFlt-1 and PlGF levels and sFlt-1/PlGF ratio in preeclamptic serum-induced HUVEC cell line.
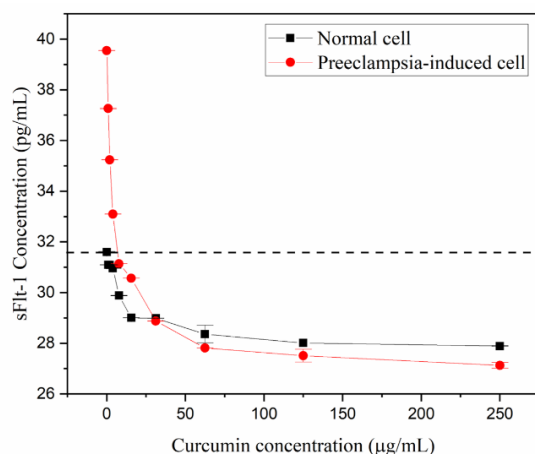
Ethical clearance

The study was conducted after the approval from Ethical Review Boards of Health Research, Faculty of Medicine and Dr. Hasan Sadikin Hospital, Bandung. All research subjects were voluntarily required to sign informed consent prior to the study.
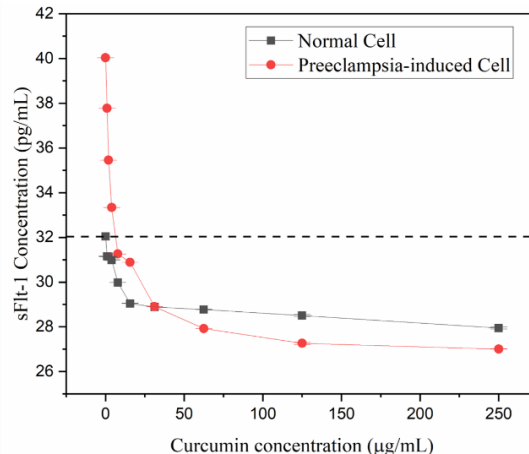
RESULTS

Effects of curcumin on sFlt-1

Initial sFlt-1 level in preeclampsia-induced HUVEC cell lines was found to be significantly higher ($p < 0.001$) than in normal cell. sFlt-1 level in preeclampsia-induced cells fell significantly ($p < 0.05$), from 39.545 to 31.135 pg/mL, after incubated for 24 h with 7.813 µg/mL curcumin (**Figure 1a**). At the same curcumin concentration, sFlt-1 level was reduced surpassing that in the untreated normal cell (31.594 pg/mL). Curcumin suppressed sFlt-1 in a concentration-dependent manner, in which sFlt-1 level was reduced by increasing curcumin concentration. A similar trend was maintained after the incubation of 48 h (**Figure 1b**).

Figure 1. Effect of different concentrations of curcumin on sFlt-1 in normal and preeclampsia-induced HUVEC cell line with 24 h (a) and 48 h (b) incubation times.

Effects of curcumin on PlGF

There was a significantly lower initial level of PlGF ($p < 0.001$) in preeclampsia induced HUVEC cell line than in normal cell. The addition of curcumin, as low as 1.953 µg/mL, contributed to a significant increase ($p < 0.05$) in PlGF level, from 2.310 to 5.450 pg/mL (**Figure 2a**). PlGF level in the preeclampsia induced HUVEC cell line (5.847 pg/mL) was close to that in the untreated normal cell line (5.888 pg/mL) when exposed with 7.8125 µg/mL curcumin and incubated for 24 h. However, for samples with 48 h incubation, higher concentration of curcumin (31.250 µg/mL) was required to reach equal PlGF levels between the preeclampsia-induced cell (6.323 pg/mL) and untreated normal cell (6.3145 pg/mL) (**Figure 2b**).
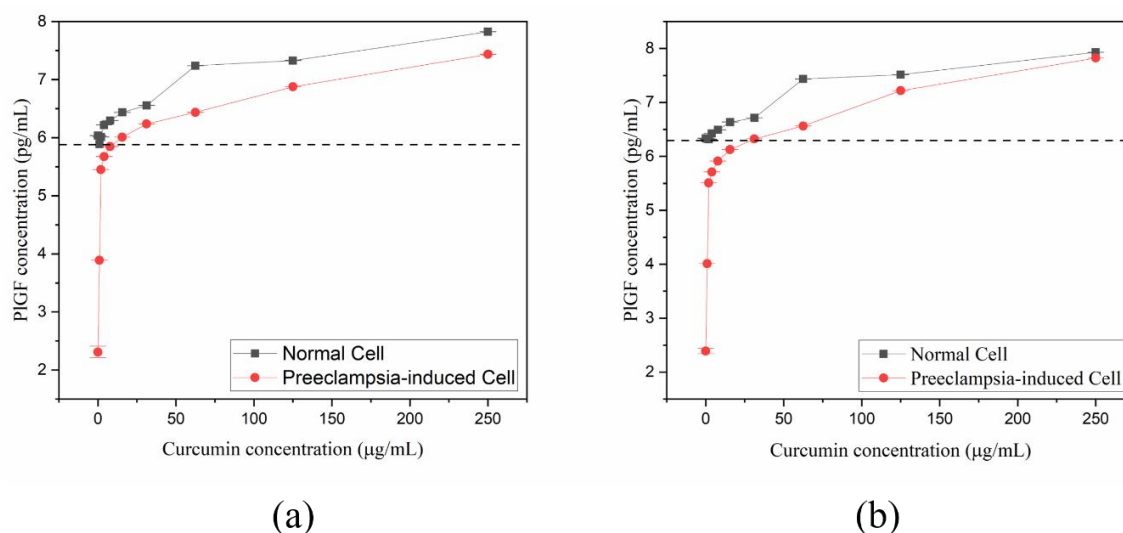
(a)   (b)

Figure 2. Effect of different concentrations of curcumin on PlGF in normal and preeclampsia induced HUVEC cell line with 24 h (a) and 48 h (b) incubation times.

Ratio of sFlt-1/PlGF
The decreasing trend of sFlt-1/PlGF ratio in response to the increasing curcumin concentration was observed in normal and preeclampsia-induced HUVEC cell lines after 24 h incubation (**Figure 3**a). The similar trend was also observed in the samples with 48 h incubation time (**Figure 3b**). Preeclamptic serum-induced HUVEC exhibited higher sFlt-1 and lower PlGF compared to those in normal cells. In samples incubated for 24 h, a significant decrease

($p<0.05$) in the sFlt-1/PlGF ratio was observed in preeclampsia-induced cell, from 17.151 to 6.466 μg/mL, after curcumin treatment with 1.953 μg/mL concentration. Similarly, the significant decrease ($p<0.05$) was observed at the same curcumin concentration in the 48 h-incubated preeclamptic cells: from 16.758 to 6.437 μg/mL. The ratio decreased surpassing that in the untreated normal cell after the addition of 7.813 μg/mL curcumin and incubation for 24 or 48 h.

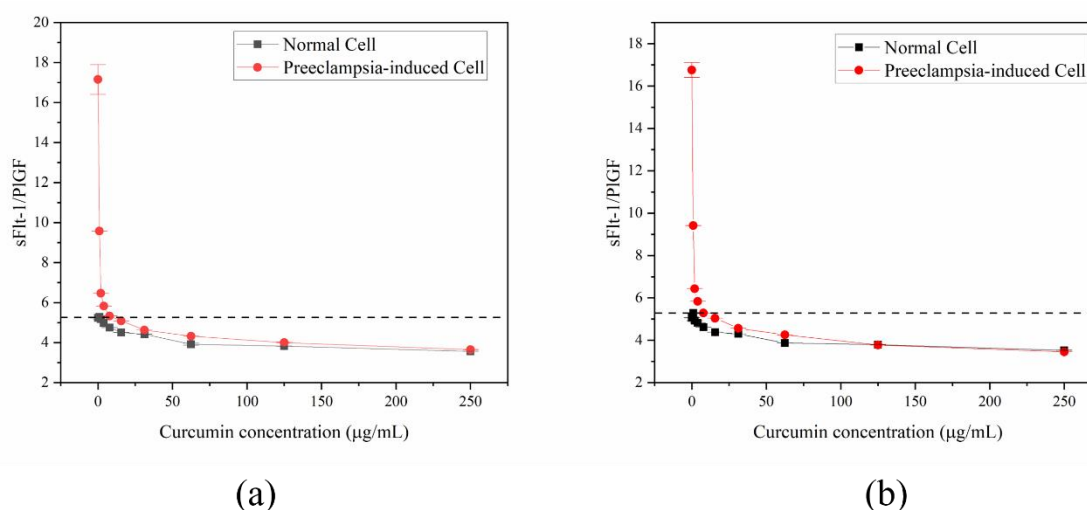(a)   (b)

Figure 3. Effect of different concentrations of curcumin on sFlt-1/PlGF in normal and preeclampsia-induced HUVEC cell lines with 24 h (a) and 48 h (b) incubation times.

DISCUSSION
The levels of sFlt-1 continue to elevate as placental hypoxia advances, and this is associated with clinical manifestations of preeclampsia, which are elevated blood pressure and proteinuria.[30] PlGF continues to reduce in severe preeclampsia which is negatively correlated with sFlt-1. Elevation of circulating sFlt-1 concentration would reduce PlGF concentration, which further results in endothelial dysfunction. This condition occurs a few weeks prior to preeclampsia, therefore can be used as a diagnostic tool of preeclampsia.[31-34] Theoretically, when elevated sFlt-1 is found in a woman without clinical manifestation of preeclampsia, the woman will have preeclampsia in the next few weeks.[33]

Referring to a recent study, sFlt-1 is responsible for oxidative stress, which generates activation of apoptosis in trophoblasts.[35] Antioxidant capacity has also been observed to decrease in maternal circulation. Treatment with antioxidants is expected to reduce oxidative stress and inhibit the release of proinflammatory mediators. In the present study, we evaluated the effect of curcumin (diferuloylmethane), which is a compound found in ground rhizomes of *Curcuma longa* L. This compound has been reported to possess antioxidant activities.[22,23]

A report suggested that mitochondria dysfunction leading to sFlt-1 production, as the result of reactive oxygen species (ROS) release, can be treated with antioxidants.[36]

In vitro and in vivo study revealed the effectiveness of mitochondrial-targeting antioxidant (resveratrol) in attenuating sFlt-1 production.[37] The effect of curcumin in suppressing mitochondrial ROS has been reported by some studies.[38,39] It is in line with our study, where the antioxidant properties of curcumin inhibit the release of sFlt-1 in preeclampsia-induced HUVEC cell line. The trends of the sFlt-1 reduction in HUVEC cells incubated with different times (24 and 48 h) are similar, indicating the antioxidant activities can be maintained after 48 h in the HUVEC cell line.

There are previous studies that show the effect of curcumin in preeclampsia. Curcumin decreased the levels of pro-inflammatory cytokines such as IL-1α, IL-6, and TNFα in monocytes culture exposed to plasma preeclamptic by affecting transcription factors of NF-kB and PPAR-γ.[24] Curcumin also improved the preeclampsia-like phenotype in rat models by reducing abnormal inflammation related to TLR4 signaling pathway.[25] Curcumin inhibited the expression of pro-inflammatory factors and macrophage infiltration in the placenta and ameliorated LPS-induced adverse pregnancy outcomes in mice by inhibiting inflammation via upregulation of phosphorylated Akt.[40]

We also observed the increase in PlGF level after the curcumin exposure in various concentrations. The sFlt-1 can bind PlGF, which means a reduction of free PlGF concentration, resulting in endothelial damage and microvascular relaxation blockage.[8] Hence, suppression of sFlt-1 by curcumin, in our study, automatically increased the level of PlGF. Nonetheless, other possibilities of endogenous PlGF enhancement by curcumin cannot be ignored. It is corroborated by the different levels of curcumin required to bring the PlGF to have a comparable level with the untreated normal cell line between that incubated for 24 h and 48 h. In samples incubated for 48 h, higher curcumin concentration is required, indicating that reduction of sFlt-1 is not the only explanation as to why the PlGF increased after the curcumin treatment. A study reported the induction of pro-angiogenic agents, VEGF, using curcumin therapy through an unknown mechanism.[41] Treatment using curcumin, therefore, can be potentially used to bring the balance between the pro-and antiangiogenic factors (in this case are PlGF and sFlt-1, respectively). The ratio of sFlt-1 and PlGF obtained after the curcumin exposure on preeclampsia-induced HUVEC cell line may portray such potential, where it is found lower than that in normal vehicle control at 7.813 μg/mL curcumin concentration.

CONCLUSION

Curcumin significantly decreased sFlt-1 and increased PlGF in preeclampsia-induced HUVEC cell line to the levels that are similar to the normal serum. These findings suggest the potential of curcumin to be used in treating preeclampsia, by restoring the balance between proangiogenic factor (PlGF) and antiangiogenic factor (sFlt-1). Further studies including in vivo studies and clinical trials of curcumin are encouraged.

REFERENCES

1. Hernández-Díaz S, Toh S, Cnattingius S. Risk of pre-eclampsia in first and subsequent pregnancies: Prospective cohort study. BMJ. 2009;339(7711):34.

2. Duley L. The Global Impact of Pre-eclampsia and Eclampsia. Semin Perinatol. 2009;33(3):130–7.

3. World Health Organization. The World Health Report 2005: Make every mother and child count. World Heal Rep. 2005.

4. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet. 2008; 371:75–84.

5. McClure J, Cooper G, Cantwell R, Clutton-Brock T, OBE GC. Saving Mothers' Lives: Reviewing maternal deaths to make motherhood safer 2006-2008. BJOG An Int J Obstet Gynaecol. 2011;118(11):127–32.

6. Staff AC, Benton SJ, Von Dadelszen P, Roberts JM, Taylor RN, Powers RW, et al. Redefining preeclampsia using placenta-derived biomarkers. J Am Heart Assoc. 2013;61(5):932–42.

7. Chaiworapongsa T, Chaemsaithong P, Yeo L, Romero R. Pre-eclampsia part 1: Current understanding of its pathophysiology. Nat Rev Nephrol. 2014;10(8):466–80.

8. Maynard SE, Min J-Y, Merchan J, Lim K-H, Li J, Mondal S, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J Clin Invest [Internet]. 2003 Mar 1;111(5):649–58. Available from: http://www.jci.org/articles/view/17189

9. Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP. Soluble Endoglin and Other Circulating Antiangiogenic Factors in Preeclampsia. N Engl J Med. 2006;355(10):992–1005.

10. Karumanchi SA, Epstein FH. Placental ischemia and soluble fms-like tyrosine kinase 1: Cause or consequence of preeclampsia? Kidney Int. 2007;71(10):959–61.

11. Kendall RL, Thomas KA. Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. Proc Natl Acad Sci U S A. 1993;90(22):10705–9.

12. Lu F, Longo M, Tamayo E, Maner W, Al-Hendy A, Anderson GD, et al. The effect of over-expression of sFlt-1 on blood pressure and the occurrence of other manifestations of preeclampsia in unrestrained conscious pregnant mice. Am J Obstet Gynecol. 2007;196(4): 396.e1-396.e7.

13. Verlohren S, Herraiz I, Lapaire O, Schlembach D, Moertl M, Zeisler H, et al. The sFlt-1/PlGF ratio in different types of hypertensive pregnancy disorders and its prognostic potential in preeclamptic patients. Am J Obstet Gynecol. 2012;206(1):58. e1-58. e8.

14. Villa PM, Hämäläinen E, Mäki A, Räikkönen K, Pesonen AK, Taipale P, et al. Vasoactive agents for the prediction of early- and late-onset preeclampsia in a high-risk cohort. BMC Pregnancy Childbirth. 2013; 13:1–10.

15. Rana S, Powe CE, Salahuddin S, Verlohren S, Perschel FH, Levine RJ, et al. Angiogenic factors and the risk of adverse outcomes in women with suspected preeclampsia. Circulation. 2012;125(7):911–9.

16. Verlohren S, Herraiz I, Lapaire O, Schlembach D, Zeisler H, Calda P, et al. New gestational phase-specific cutoff values for the use of the soluble fms-like tyrosine kinase-1/placental growth factor ratio as a diagnostic test for preeclampsia. Hypertension. 2014;63(2):346–52.

17. Yiyenoğlu ÖB, Uğur MG, Özcan HÇ, Can G, Öztürk E, Balat Ö, et al. Assessment of oxidative stress markers in recurrent pregnancy loss: A prospective study. Arch Gynecol Obstet. 2013;289(6):1337–40.

18. Rajimakers M, Peters W, Steegers E, L P. Amino thiols, detoxification and oxidative stress in pre-eclampsia and other disorders of pregnancy. Curr Pharm Des. 2005;11(6):711–34.

19. Tenório MB, Ferreira RC, Moura FA, Bueno NB, Goulart MOF, Oliveira ACM. Oral antioxidant therapy for prevention and treatment of preeclampsia: Meta-analysis of randomized controlled trials. Nutr Metab Cardiovasc Dis [Internet]. 2018 Sep;28(9):865–76. Available from: https://linkinghub.elsevier.com/retrieve/pii/S09394 7531830190X

20. Lim SN, Cheung PCK, Ooi VEC, Ang PO. Evaluation of antioxidative activity of extracts from a brown seaweed, Sargassum siliquastrum. J Agric Food Chem. 2002;50(13):3862–6.

21. Kayano SI, Kikuzaki H, Fukutsuka N, Mitani T, Nakatani N. Antioxidant activity of prune (Prunus domestica L.) constituents and a new synergist. J Agric Food Chem. 2002;50(13):3708–12.

22. Aggarwal BB, Kumar A, Bharti AC. Anticancer potential of curcumin: Preclinical and clinical studies. Anticancer Res. 2003;23(1 A):363–98.

23. Duvoix A, Blasius R, Delhalle S, Schnekenburger M, Morceau F, Henry E, et al. Chemopreventive and therapeutic effects of curcumin. Cancer Lett. 2005;223(2):181–90.

24. Rahardjo B, Widjajanto E, Sujuti H, Keman K. Curcumin decreased level of proinflammatory cytokines in monocyte cultures exposed to preeclamptic plasma by affecting the transcription factors NF-κB and PPAR-γ. Biomarkers Genomic Med. 2014;6(3):105–15.

25. Gong P, Liu M, Hong G, Li Y, Xue P, Zheng M, et al. Curcumin improves LPS-induced preeclampsia-like phenotype in rat by inhibiting the TLR4 signaling pathway. Placenta. 2016 May; 41:45–52.

26. Gunardi JI, Mose J, Satari MH, Anwar AD, Fauziah PN, Triyuli. Effects of papua ant nests (Myrmecodia pendens) on level of sFlt-1, PlGF, MDA and NO in preeclampsia-induced HUVEC cell line. Int J PharmTech Res. 2016;9(6):424–35.

27. Neale D, Demasio K, Illuzi J, Chaiworapongsa T, Romero R, Mor G. Maternal serum of women with pre-eclampsia reduces trophoblast cell viability: evidence for an increased sensitivity to Fas-mediated apoptosis. J Matern Neonatal Med [Internet]. 2003 Jan 7;13(1):39–44. Available from: http://www.tandfonline.com/doi/full/10.1080/jmf.1 3.1.39.44

28. Munaut C, Lorquet S, Pequeux C, Blacher S, Berndt S, Frankenne F, et al. Hypoxia is responsible for soluble vascular endothelial growth factor receptor-1 (VEGFR-1) but not for soluble endoglin induction in villous trophoblast. Hum Reprod. 2008;23(6):1407–15.

29. Basyir V, Fauziah P, Yanwirasti, Johannes M. Effect of Lycopene on Level of Malondialdehid (MDA) in Preeclampsia-Induced Placental Trophoblast Cells. Int J PharmTech Res. 2017;10(2):103–8.

30. Tsatsaris V, Goffin F, Munaut C, Brichant JF, Pignon MR, Noel A, et al. Overexpression of the Soluble Vascular Endothelial Growth Factor Receptor in Preeclamptic Patients: Pathophysiological Consequences. J Clin Endocrinol Metab. 2003;88(11):5555–63.

31. Cunningham F, Leveno K, Bloom S, Hauth J, Rause D. Williams Obstetrics. 23rd ed. New York: Mc Graw Hill; 2010.

32. Lam C, Lim KH, Karumanchi SA. Circulating angiogenic factors in the pathogenesis and prediction of preeclampsia. Hypertension. 2005;46(5):1077–85.

33. Stepan H, Geide A, Faber R. Soluble fms-like Tyrosine Kinase 1. N Engl J Med. 2004;351(21):2241–2.

34. Chen Y. Novel angiogenic factors for predicting preeclampsia: sFlt-1, PlGF, and soluble endoglin. Open Clin Chem J. 2009;2(January 2009):1–6.

35. Jiang Z, Zou Y, Ge Z, Zuo Q, Huang SY, Sun L. A role of sFlt-1 in oxidative stress and apoptosis in human and mouse pre-eclamptic trophoblasts. Biol Reprod. 2015;93(3):1–7.

36. Jena MK, Sharma NR, Petitt M, Maulik D, Nayak NR. Pathogenesis of Preeclampsia and Therapeutic Approaches Targeting the Placenta. Biomolecules [Internet]. 2020 Jun 24;10(6):953. Available from: https://www.mdpi.com/2218-273X/10/6/953

37. Hastie R, Brownfoot FC, Pritchard N, Hannan NJ, Cannon P, Nguyen V, et al. EGFR (Epidermal Growth Factor Receptor) Signaling and the Mitochondria Regulate sFlt-1 (Soluble FMS-Like Tyrosine Kinase-1) Secretion. Hypertension [Internet]. 2019 Mar;73(3):659–70. Available from: https://www.ahajournals.org/doi/10.1161/HYPERT ENSIONAHA.118.12300

38. Zhang J, Bai K wen, He J, Niu Y, Lu Y, Zhang L, et al. Curcumin attenuates hepatic mitochondrial dysfunction through the maintenance of thiol pool, inhibition of mtDNA damage, and stimulation of the mitochondrial thioredoxin system in heat-stressed broilers. J Anim Sci [Internet]. 2018 Apr 3;96(3):867–79. Available from: https://academic.oup.com/jas/article/96/3/867/493 9326

39. Gibellini L, Bianchini E, De Biasi S, Nasi M, Cossarizza A, Pinti M. Natural Compounds Modulating Mitochondrial Functions. Evidence-Based Complement Altern Med [Internet]. 2015; 2015:1–13. Available from: http://www.hindawi.com/journals/ecam/2015/5272 09/

40. Zhou J, Miao H, Li X, Hu Y, Sun H, Hou Y. Curcumin inhibits placental inflammation to ameliorate LPS-induced adverse pregnancy outcomes in mice via upregulation of phosphorylated Akt. Inflamm Res. 2017;66(2):177–85.

41. Basak S, Srinivas V, Mallepogu A, Duttaroy AK. Curcumin stimulates angiogenesis through VEGF and expression of HLA-G in first-trimester human placental trophoblasts. Cell Biol Int [Internet]. 2020 May;44(5):1237–51. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/cbi n.11324