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Syzygium Cumini Reduces Vcam-1 and Icam-1 Expression in Endothelial Cells Induced by Plasma from Preeclamptic Patients

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Abstract

The present study sought to investigate a role for Syzygium cumini on VCAM-1 and ICAM-1 expression in endothelial cells induced by plasma from preeclamptic patients. Endothelial cells were obtained from human umbilical vascular endothelial cells. At confluency, endothelial cells were divided into five groups, which included control (untreated), endothelial cells exposed to 2% plasma from preeclamptic patients (PP), endothelial cells exposed to PP in the presence of ethanolic extract of Syzygium cumini (PP + SC) at the following three doses: 100; 200; and 400 ppm. Analysis of ICAM-1 and VCAM-2 level were done by immunohistochemistry technically. This increase in VCAM-1 was significantly ($P < 0.05$) attenuated by both the 200 and 400 ppm treatments of Syzygium cumini extract. Plasma from PP significantly increased ICAM-1 levels compared to untreated cells. This increase in ICAM-1 was significantly attenuated by the 400 ppm doses of the extract. In conclusion, Syzygium cumini extract prohibits the increase in VCAM-1 and ICAM-1 in endothelial cells induced by plasma from preeclamptic patients. Therefore this may provide a natural therapy for attenuating the adhesion molecule induced by endothelial dysfunction found in this pregnancy complication.

Keywords: Herbal medicine, pregnancy, hypertension, adheison molecules, *Syzygium Cumini*

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Introduction

Preeclampsia (PE) is a specific syndrome that is characterized by an increase in blood pressure (> 140/90 mm Hg), proteinuria, and edema [1]. This health problem is one of the causes of high maternal and perinatal morbidity and mortality with unclear etiology. Poor placental perfusion accompanied by the endothelial cell dysfunction and a disturbed balance of angiogenic factors may all contribute to this disorder. Poor placental perfusion leads to the release of soluble placental-derived antiangiogenic and inflammatory factors into the maternal circulation and subsequent endothelial dysfunction [3].

Vascular endothelial cells are activated during oxidative stress, which marked by increasing expression of adhesion molecules. Physiologically, the VCAM-1 and ICAM-1 are present in the membranes of endothelial cells in low expressions. When the stimulus presence, this molecule will upregulated [4]. In preeclampsia, this adhesion molecules levels showed significant correlations with blood pressure values, renal and liver function markers [5]. In order to inhibits this dysfunction, a new approach by herbal treatment may promise. Recently, there were limited studies regarding the application of active compound from plants for preeclampsia [6-9].

Syzygium cumini known as Java plum is a small, ovoid form, purple-red to black color when ripe fruit. This Myrtaceae family originates from Indonesia [10]. This fruit was containing a fleshy pink or almost white pulp with an astringent taste performs several biofunction as radioprotective, gastroprotective, antioxidant, and antiinflammatory [11-15]. The antioxidant action of *Syzygium cumini* fruits is better than catechin or Trolox [16]. As far as we know, there was no information in the published literature about the role of *Syzygium cumini* on adhesion molecule expression in endothelial cells. The present study sought to investigate a role for *Syzygium cumini* on VCAM-1 and ICAM-1 expression in endothelial cells induced by plasma from preeclamptic patients.

Material and Methods

Syzygium cumini extraction

Syzygium cumini was obtained from the Depok region, West Java, Indonesia (6° 22' 21 S 106° 49' 39 E)**.** Drying was done by distinguishing fruit from seed then dry in oven (80^{o}C) or

sunlight. Extraction was done by blending fruit using juicer to get 100 g powder. A hundred grams of powder was then added to 900 ml 70% ethanol in 1 liter Erlenmeyer flask. The solution was allowed to evaporate overnight. The next day, upper layers were collected and connected to an evaporation apparatus. Extracted specimen were stored in -40^{o}C.

Collection of plasma preeclamptic patients

Prior to blood collection all patients signed the informed consent which approved by Health Research Ethics Committee of the Faculty of Medicine Brawijaya University. Clinical characteristics of preeclamptic patient were systolic blood pressure (150 mmHg), diastolic blood pressure (110 mmHg), and proteinuria (3+) on dispstick. Plasma was isolated from 10 mL of whole blood that was collected from preeclampsia patients using citrate as an anticoagulant. For this purpose, blood was centifuge at 1000g, 10 minutes for 4^{o}C. Plasma was used in cell culture experiments as detailed below.

Endothelial cells isolation and culture

Human umbilical vein endothelial cells (HUVECs) were obtained from pregnant women who met the following criteria: a healthy pregnancy (hemoglobin level ≥ 10 g/dl) accompanied by section cesarean delivery (38 weeks of gestation). Immediately postpartum, 20 cm of the umbilical cord was placed in Cord solution (*Hank's Balance salt solution* (HBSS) 100 ml, gentamycine, *sodium hydrogen bicarbonate* dan *phenol red* 4 ml, HEPES solution 2 ml, *deionized water*) from 4^{o}C and kept cold until endothelial cells were isolated. Endothelial cell isolation was performed no greater that 12 hours after delivery.

Umbilicus cleared of tissue and blood clot with alcohol. Cannula inserted ± 1.5 cm deep at one end of the vein, and tied as tightly as possible. Veins were washed with PBS using a pipette to clean, then end without cannula is clamped tightly. Eight milliliters of collagenase inserted into the vein with the syringe still attached and clamped, then held by hand for ± 8 minutes. The solution obtained is then centrifuged at 1000 rpm for 8 minutes. Pellet was homogenized with culture medium (medium 199, gentamycin, bicarbonate phenol red, glutamine) plus newborn calf plasma). Pellets were inserted into the well 24 pieces that have been coated with gelatin, were grown in an incubator until the monolayer. Cell cultures are washed every day with plasma free media and new media is replaced. Cell were allowed to

grow to confluence at 37^{o}C 95%O_2 5%CO_2. Once confluent, cells wells was divided into 4 replicated including an untreated group; cells treated with 2% plasma preeclamptic patients (PP); PP + the ethanolic extract from *Syzygium cumini* (SC, 100 ppm); PP + SC (200 ppm); and PP + SC (400 ppm) [17].

Plasma and extract treatment

Endothelial cell cultures were incubated at 37^{0}C for 72 hours in DMEM containing 20% FBS (v / v), with the addition of 2% plasma of preeclampsia. As control cells were cultured without added plasma preeclampsia. Then the cell cultures were washed 3 times with PBS, incubated with *Syzygium cumini* extract (100 ppm, 200 ppm and 400 ppm) at a temperature of 37^{0}C.

Analysis of VCAM-1 and ICAM-1

Levels of VCAM-1 and ICAM-1 were determined in the endothelial cell culture using the immunohistochemistry method. The procedure was performed according the previous studies.

Ethics

This study was approved by the Health Research Ethics Committee of the Faculty of Medicine Brawijaya University.

Statistical analysis

Data are presented as mean ± SD and differences between groups were analyzed using one way ANOVA with SPSS 17.0 statistical package. The post Hoc test was used if the ANOVA was significant. $P < 0.05$ was considered statistically significant.

Results

Table 1 presents the VCAM-1 and ICAM-1 levels in the cell culture media from each endothelial cell experimental group. The level of VCAM-1 was significantly greater in the PP group compared to the untreated control group ($P < 0.05$). Out of the 100 ppm, 200 ppm, and 400 ppm doses of *Syzygium cumini* extract, only the two highest doses significantly prevented PP-induced increase in VCAM-1 level. There was no significant different between the effects

of these two highest doses. The ICAM-1 levels were significantly higher in the PP group compared to the untreated group ($P < 0.05$). These increased levels of ICAM-1 in the PP group were significantly reduced by 400 ppm administration of *Syzygium cumini* extract.

Table 1. Level of VCAM-1 and ICAM-1 of endothelial cells induced by plasma preeclamptic patients

			PP + *Syzygium cumini*		
Level	Control	PP	100 ppm	200 ppm	400 ppm
VCAM-1 (%)	9.30 ± 4.36	87.53 ± 7.58[a]	81.73 ± 7.72[a]	67.33 ± 10.14[abc]	61.87 ± 9.97[abc]
ICAM-1 (%)	11.65 ± 1.16	85.78 ± 10.10[a]	67.58 ± 12.61[a]	53.46 ± 17.56[a]	41.15 ± 16.70[ab]

Note: values are presented as mean ± SD; [a]$p<0.05$; in comparison with control group; [b]$p<0.05$; in comparison with PP group; [c]$p<0.05$; in comparison with first dose administered group; VCAM-1: vascular cell adhesion molecule-1; ICAM-1: intercellular adhesion molecule-1; PP: plasma preeclamptic patients; ppm: part per million; %: percentage.

Discussion

Cellular forms of adhesion molecules mediate specific steps of leukocyte–endothelial cell interaction. The VCAM-1 functions as a transmembrane receptor for the vascular endothelial cell membrane and recruits leukocyte to the sites of inflammation. The key function of ICAM-1 is the regulation of leukocyte migration to the endothelium and leukocyte activation [18]. We found that the level of VCAM-1 and ICAM-1 were significantly higher in the media from endothelial cells treated with plasma from preeclamptic women compared with untreated cells ($P < 0.05$). Our finding showed that plasma from preeclamptic women able to upregulates the VCAM-1 and ICAM-1 of endothelial cells. This increased level of VCAM-1 and ICAM-1 indicate alterations in cellular functions [19, 20]. The source of the inflammatory stimulus during preeclampsia seems to be the placenta, which driven by cytokine [21]. These inflammatory signals will induce the synthesis, expression, and shedding of VCAM-1 or ICAM-1 in endothelial cells [19, 20].

Only the two highest doses of *Syzygium cumini* extract significantly prevented PP-induced increase in VCAM-1 level. For ICAM-1, these increased levels in the PP group were

significantly reduced by the highest dose administration of *Syzygium cumini* extract. We hypothesized that the antioxidant of *Syzygium cumini* inhibits upregulation of VCAM-1 and ICAM-1 of endothelial cells [22-24]. For the difference effect from VCAM-1 or ICAM-1, there is evidence that there is antioxidant-sensitive transcriptional regulatory mechanism for VCAM–1 gene expression. Cytokine-activated VCAM–1 gene expression in cultured human umbilical vein endothelial cells may be almost completely inhibited by the addition of antioxidants. In contrast, the addition of antioxidants does not suppress the cytokine-induced ICAM–1 gene expression [25].

In conclusion, *Syzygium cumini* extract prohibits the increase in VCAM-1 and ICAM-1 in endothelial cells induced by plasma from preeclamptic patients. Therefore, in clinical setting this may provide an herb therapy for balancing the VCAM-1 and ICAM-1 induced by endothelial dysfunction found in this pregnancy complication.

Declaration of interest

All author(s) declare(s) that there is no conflict of interests regarding the publication of this article.

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1. Davison JM, Homuth V, Jeyabalan A, Conrad KP, Karumanchi SA, Quaggin S, Dechend R, Luft FC. New aspect in the pathophysiology of preeclampsia. J Am Soc Nephrol. 2004;15(9):2440-8.
2. Noris M, Perico N, Remuzzi G. Mechanisms of disease: pre-eclampsia. Nature Clin Pract Nephrol. 2005;1(2):98-114.
3. Sedeek M, Gilbert JS, Lamarca BB, Sholook M, Chandler DR, Wang Y, Granger JP. Role of reactive oxygen species in hypertension produced by reduced uterine perfusion in pregnant rats. Am J Hypertens. 2008;21(10):1152-6.
4. Molvarec A, Szarka A, Walentin S, Beko G, Karádi I, Prohászka Z, Rigó J Jr. Serum heat shock protein 70 levels in relation to circulating cytokines, chemokines, adhesion molecules and angiogenic factors in women with preeclampsia. Clin Chim Acta. 2011;412(21-22):1957-62.

5. Szarka A, Rigo J Jr, Lazar L, Beko G, Molvarec A. Circulating cytokines, chemokines and adhesion molecules in normal pregnancy and preeclampsia determined by multiplex suspension array. BMC Immunol. 2010;11:59.
6. de Souza CO, Peraçoli MT, Weel IC, Bannwart CF, Romão M, Nakaira-Takahagi E, Medeiros LT, Silva MG, Peraçoli JC.Hepatoprotective and anti-inflammatory effects of silibinin on experimental preeclampsia induced by l-NAME in rats. Life Sci. 2012;91(5-6):159-65.
7. Takei H, Nakai Y, Hattori N, Yamamoto M, Takeda S, Yamamoto M, Arishima K. The herbal medicines Saireito and Boiogito improve the hypertension of pre-eclamptic rats induced by Nω-Nitro-l-arginine methyl ester. Phytomedicine. 2007;14(9):591-600.
8. Giorgi VSI, Peracoli MT, Peracoli JC, Witkin SS, Bannwart-Castro CF. Silibinin modulates the NF-κb pathway and pro-inflammatory cytokine production by mononuclear cells from preeclamptic women. J Reprod Immunol. 2012;95(1-2):67-72.
9. Takei H, Nakai Y, Hattori N, Yamamoto M, Kurauchi K, Sasaki H, Aburada M. The herbal medicine Toki-shakuyaku-san improves the hypertension and intrauterine growth retardation in preeclampsia rats induced by Nω-nitro-L-arginine methyl ester. Phytomedicine. 2004;11(1):43-50.
10. Veigas JM, Narayan MS, Laxman PM, Neelwarne B. Chemical nature, stability and bioefficacies of anthocyanins from fruit peel of Syzygium cumini Skeels. Food Chem. 2007;105:619-27.
11. Chaturvedi A, Kumar MM, Bhawani G, Chaturvedi H, Kumar M, Goel RK. Effect of ethanolic extract of Eugenia jambolana seeds on gastric ulceration and secretion in rats. Indian J Physiol Pharmacol. 2007;51(2):131-40.
12. Jagetia GC, Baliga MS. S. cumini (Jamun) reduces the radiation-inducedDNA damage in the cultured human peripheral blood lymphocytes: a preliminary study. Toxicol Lett. 2002;132(1):19-25.
13. Banerjee A, Dasgupta N, De B. In vitro study of antioxidant activity of S. cumini fruit. Food Chemistry. 2005;90(4):727-33.
14. Benherlal PS, Arumughan C. Chemical composition and in vitro antioxidant studies of S. cumini fruit. J Sci Food Agric. 2007;87(14):2560-9.
15. Chaudhuri AKN, Pal S, Gomes A, Bhattacharya S. Anti-inflammatory and related actions of S. cumini seed extract. Phytotherapy Research. 1990;4:5-10.
16. Benherlal PS, Arumughan C. Chemical composition and in vitro antioxidant studies on Syzygium cumini fruit. J Sci Food Agric. 2007;87(14):2560-9.
17. Veigas JM, Shrivasthava R, Neelwarne B. Efficient amelioration of carbon tetrachloride induced toxicity in isolated rat hepatocytes by Syzygium cumini Skeels extract. Toxicol In Vitro. 2008;22(6):1440-6.
18. Krieglstein CF, Granger DN. Adhesion molecules and their role in vascular disease. Am J Hypertens. 2001;14(6 Pt 2):44S-54S.

19. Blankenberg S, Barbaux S, Tiret L. Adhesion molecules and atherosclerosis. Atherosclerosis. 2003;170(2):191-203.
20. Jenny NS, Arnold AM, Kuller LH, Sharrett AR, Fried LP, Psaty BM, Tracy RP. Soluble intracellular adhesion molecule-1 is associated with cardiovascular disease risk and mortality in older adults. J Thromb Haemost. 2006;4(1):107-13.
21. Madazli R, Aydin S, Uludag S, Vildan O, Tolun N. Maternal plasma levels of cytokines in normal and preeclamptic pregnancies and their relationship with diastolic blood pressure and fibronectin levels. Acta Obstet Gynecol Scand. 2003;82(9):797-802.
22. Banerjee A, Dasgupta N, De B. In vitro study of antioxidant activity of Syzygium cumini fruit. Food Chemistry. 2005; 90(4):727-33.
23. Veigas JM, Narayan MS, Laxman PM, Neelwarne B. Chemical nature, stability and bioefficacies of anthocyanins from fruit peel of Syzygium cumini Skeels. Food Chemistry. 2007;105(2):619-27.
24. Arun R, Prakash MVD, Abraham SK, Premkuma K. Role of Syzygium cumini seed extract in the chemoprevention of in vivo genomic damage and oxidative stress. J Ethnopharmacol. 2011;134(2):329-33.
25. Marui N, Offermann MK, Swerlick R, Kunsch C, Rosen CA, Ahmad M, Alexander RW, Medford RM. Vascular cell adhesion–1 (VCAM-1) gene transcription and expression are regulated through an antioxidant-sensitive mechanism in human vascular endothelial cells. J Clin Invest. 1993;92(4):1866-74.