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Anti-angiogenic Effect of Black Rice Bran (*Oryza Sativa* L. 'Sembada Hitam') on Soluble Fms-Like Tyrosine Kinase and Placental Growth Factor in Preeclampsia

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ABSTRACT

Preeclampsia is a multisystem disorder of pregnancy that contributes to morbidity and mortality worldwide. Soluble FMS-like tyrosine kinase-1 (sFlt-1) is considered as an etiologic factor of endothelial damage in preeclampsia. Imbalance of sFlt-1 and PlGF could cause failure in trophoblastic invasion and physiological remodeling of spiral artery, and ultimately placental hypoxia. This study aimed to observe anti-angiogenic properties of black rice bran on preeclampsia through measurement of sFlt-1 and PlGF. Serum was collected from pregnant women at 28-34 weeks of gestational age with preeclampsia, and normal preeclampsia as control. Level sflt-1 and PGIF was quantified with enzyme linked immunosorbent assay (ELISA). Absorbance was read at 450 nm wavelength. Extract of black rice bran has anti-angiogenic properties by significantly decreasing sFlt-1 as well as increasing PlGF in preeclampsia-induced HUVEC. This makes black rice bran a promising agent which can be further used in preeclampsia treatment.

Keywords: Black rice bran, placental hypoxia, preeclampsia, PlGF, sFlt-1

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INTRODUCTION

Preeclampsia is a multisystem disorder that complicates 3%–8% of pregnancies in Western countries and contributes to morbidity and mortality worldwide[1]. Preeclampsia is characterized by an increased blood pressure more than 140/90 mmHg and proteinuria more than 300 mg/24 h (dipstik +1) with or without edema. Preeclampsia/eclampsia contributed to about 13% maternal mortality in Indonesia[2]. Its incidences in Hasan Sadikin General Hospital (RSHS) was approximately from 4 to 10% in 2013–2015, which contributes to 10,4% maternal mortality in the hospital[3].

Soluble FMS-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF) are considered as etiologic factors in endothelial damage in preeclampsia. Elevation of sFlt-1 concentration was found weeks before clinical onset of hypertension and proteinuria[4-6]. PlGF., a type of vascular endothelial growth factor (VEGF), is a dimeric glycoprotein amino-acid residue which is an important local mediator in angiogenesis. Alteration of its circulating concentration may affect the balance of placental vessel angiogenesis; thus, its presence is frequently correlated with preeclampsia due to its profile of abnormality in placental development[4,7,8]. Biochemical imbalance in preeclampsia occurs with an increase of oxidative stress and lipoperoxidation and at the same time, a deficient antioxidant protection. Lipid peroxides, as products of an altered oxidative stress, are involved in endothelial cell injury, vasoconstriction and imbalance between thromboxane and prostacyclin[7]. Levels of plasma and erythrocytic malondialdehyde (MDA), a marker of lipid peroxidation in normotensive pregnant women, has been documented in women with established preelampsia[8]. Thus, antioxidants is considered as important approach to compensate lipid which is associated with preeclampsia.

The exogenous antioxidants are found in food and medicinal plants, such as fruits, vegetables, cereals, mushrooms, beverages, flowers, spices and traditional medicinal herbs. The industries processing agricultural by-products are also potentially important sources of natural antioxidants. Thus we would like to observe antioxidant actvities in agricultural waste. Black rice *'Sembada Hitam'* is a black rice cultivar in Yogyakarta, which is planted in Sleman and Bantul region, Indonesia[9] Biological and antioxidant activity of rice is mainly localized in its bran (the outer layer)[10, 11]. Black rice bran contains various phenolic acids (that is, ferulic acid, p-coumaric acid, vanillic acid, p-hydroxybenzoic, gallic acid, and protocatechuic acid), and ferulic acid has been identified as the predominant phenolic antioxidant[10,12]. All these findings raises interest to observe antiangiogenic and antioxidant properties of black rice bran on preeclampsia through measurement of sFlt-1, PIGF and MDA.

MATERIAL AND METHODS

Study design

This was true experimental study with posttest only control group design. Research subjects were pregnant women at 28-34 weeks of gestational age consisting of women with preeclampsia and normal pregnancy as controls. This study was performed in Laboratory of Molecular Genetics, Faculty of Medicine, Universitas Padjajaran, from January 2018 to January 2019. Blood samples were collected from patients admitted to Department of Obstetrics and Gynecology, Hasan Sadikin General Hospital (RSHS), and informed consent was obtained. This study was approved by Ethics Committee of Faculty of Medicine/RSHS.

Black rice bran extraction

Black Rice bran '*Sembada Hitam*' was obtained by scraping the outer layer of whole black rice. Rice bran was sieved with a 60-mesh sieve and then 10 mg of rice bran was extracted using 100 ml of ethanol acidified with HCl 1N (ethanol:HCl 1N, 85:15) by maceration for 48 hours at room temperature and stirring occasionally. The extract was filtered using Whatman No.1 paper. The filtrate was remacerated with 50 ml of solvent containing ethanol:HCl 1 N (85:15) twice for overnight. The extract was evaporated with a fan until it formed a paste.

HUVEC cell culture

HUVEC (human umbilical vein endothelial cell) cells were cultured in Roswell Park Memorial Institue (RPMI) 1640 medium containing 20% (v/v) sample (normal or preeclamptic serum)[13], 10% endothelial supplement and antibiotic-antimycotic (1% penicillin G-streptomycin Solution Stabilised and 1% Fungisone Amphotericin B) and 1% gentamisin. Cells were incubated at 37˚C 5% CO_2 [14-16].

Measurement of Lethal Concentration (LC_{50})

LC_{50} of black rice bran was measured with BSLT (Brine Shrime Lethality Test).[17] Cells (6x10^5 cell/mL) was added with black rice bran extract in various concentrations. Absorbance were read at 517 nm wavelength. LC_{50} was measured with following formula:

Measurement of sFlt-1 and PlGF levels

Cells (6x10^5 cell/mL) were placed into 96-wells microplate, and incubated at 37˚C 5% CO_2 (v/v). Futhermore, wells were washed with PBS (phosphate buffered saline) 37^{0}C three or four times to discard remaining medium and unattached cells. Black rice bran in various concentrations (0; 0.977; 1.953; 3.906; 7.813; 15.625; 31.25; 62.5; 125; 250 µg/mL) was placed into wells and incubated for 24 to 48 hours at 37˚C 5% CO_2 (v/v). Levels of sFlt-1 and PlGF were quantified with ELISA CLOUD-CLONE. Absorbance was read at 450 nm wavelength[15, 16].

Data analysis

Data were analyzed with two way- analysis of varians (ANOVA) and continued with post hoc Dunnett using Statistical Package for the Social Sciences (SPSS) 21.0 Windows 213045 2019.

Ethical clearance

This study was approved by the Universitas Padjadjaran Bioethics Review Board (no.11/UN6.KEP/EC/2018). Informed consent was obtained from each respondent before information was collected.

RESULTS

As shown in Table 1, treatment with black rice bran reduced levels of sFlt-1 in preeclampsia-induced HUVEC. Starting from 15.625 µg/ml, black rice bran significantly reduced sFlt-1 level ($p<0.05$) after 24 and 48 hours of incubation.

Table 1. Levels of sFlt-1 in preeclampsa-induced Human umbilical vein endothelial cell (HUVEC) treated with black rice bran

Treatment (black rice bran)	Normal pregnancy		Preeclampsia		p-value
	24 hours incubation	48 hours incubation	24 hours incubation	48 hours incubation	
Control	30,5935 (0.0445)* (a)*	30,0430 (0.0014) (b)	40,5450 (0.0014) (c)	39,5460 (0.0) (c)	<0.001
0.977	30,0935 (0,0078) (a)	30,1500 (0.0071) (b)	38,2600 (0,0354) (c)	38,2850 (0.00) (c)	0.774
1.953	30,0870 (0.0014) (a)	30.1390 (0,0071) (b)	37,2370 (0.0071) (c)	37,2420 (0.00) (c)	0.187
3.906	29,9595 (0.0092) (a)	29.9895 (0,0035) (b)	36,0950 (0.0085) (c)	33,1010 (0.00) (c)	0.013
7.813	28.8815 (0,0134) (a)	28,9785 (0,0092) (b)	31.1350 (0,0014) (c)	31.1360 (0,00) (c)	0.001
15.625	28.0040 (0,0028) (a)	28,0470 (0,0028) (b)	30.5660 (0,0056) (c)	30.5700 (0,00) (c)	<0.001
31.25	27.9875 (0,0064) (a)	27.8870 (0,0028) (b)	28.8750 (0,0056) (b)	28.8790 (0,00) (b)	<0.001
62.5	27.3600 (0,4936) (ab)	27,7765 (0,0078) (a)	27.8125 (0,0007) (b)	27.8130 (0,00) (b)	<0.001
125.0	27.0100 (0,00) (a)	27.5025 (0.0686) (ab)	27.5120 (0,3536) (b)	27.2620 (0,00) (b)	<0.001
250.0	25.8890 (0,0184) (a)	26.9450 (0.0650) (a)	27.1245 (0,1605) (b)	27.0110 (0,00) (b)	<0.001

Data are presented as mean values. Different letters (a-c) in the same row indicates significant difference among treatment, analyzed with post hoc Dunnett test ($p<0.05$).

Black rice bran increased the level of PIGF in preeclampsia-induced HUVEC in a concentration-dependent manner (Table 2). At concentration of 15.625 µg/ml, the PIGF level in preeclampsia-induced HUVEC after 24 (6.0105 pg/ml) and 48 hours (6.1260 pg/ml) of incubation was comparable to normal (6.07 pg/ml) ($p<0.05$).

Table 2. Levels of PIGF in preeclampsa-induced HUVEC treated with black rice bran

Treatment (black rice bran)	Normal pregnancy		Preeclampsia		p-value
	24 hours incubation	48 hours incubation	24 hours incubation	48 hours incubation	
Control	6.07 (0,0078)* (a)*	6.33 (0.0049) (b)	2.3100 (0.1414) (c)	2.3900 (0.0707) (c)	<0.001
0.977	5.70 (0.0014) (a)	5.80 (0.0014) (a)	3.8905 (0.0021) (b)	3.8905 (0.0021) (c)	0.599
1.953	6.20 (0.0071) (a)	6.24 (0.0049) (b)	5.4500 (0.0014) (c)	5.5075 (0.0007) (d)	0.101
3.906	6.75 (0,0021) (a)	6.20 (0,0014) (b)	5.6750 (0,00452) (c)	5.7095 (0,0021) (d)	0.005
7.813	6.30 (0.0085) (a)	6.49 (0.0028) (b)	5.8470 (0,0071) (c)	5.9130 (0,0028) (d)	<0.001
15.625	6.35 (0,0092) (a)	6.63(0,0056) (b)	6.0105 (0,0021) (c)	6.1260 (0,0014) (d)	<0.001
31.25	6.55 (0,0064) (a)	6.71(0,0028) (b)	6.2360 (0,0028) (c)	6.3230 (0,0028) (d)	<0.001
62.5	7.38 (0,0035) (a)	7.47(0,0042) (b)	6.4365 (0,0021) (c)	6.5630 (0,0028) (d)	<0.001
125.0	7.35 (0,0049) (a)	7.77 (0,0021) (b)	6.8770 (0,0014) (c)	7.2185 (0,0007) (d)	<0.001
250.0	7.05 (0.0049) (a)	8.02(0.0021) (b)	7.4355 (0.0049) (c)	7.8240 (0.0014) (a)	<0.001

Data are presented as mean values. Different letters (a-c) in the same row indicates significant difference among treatment, analyzed with post hoc Dunnett test ($p<0.05$)

DISCUSSION

Elevated sFlt-1 concentration might continue as placental hypoxia advances, and this is in line with clinical manifestation of preeclampsia, including high blood pressure and proteinuria[18]. Elevation of circulating sFlt-1 concentration reduce PlGF concentration until endothelial dysfunction ensues. This is documented a few weeks prior to preeclampsia, which can be further used as a diagnostic tool of preeclampsia[19-22]. When elevated sFlt-1 is found in a woman without clinical manifestation of preeclampsia, the woman will likely develop preeclampsia within a few weeks[21]. Levels of plasma and erythrocytic MDA, has also been documented in women with established preelampsia[8]. Thus, antioxidants is considered as important approach to compensate lipid which is associated with preeclampsia.

In the present study, black rice bran exhibited antiangiogenesis properties by decreasing sFlt-1 and increasing PIGF. Likewise, it also exhibited a very strong antioxidant properties. Treatment of antioxidants has been proposed in preeclampsia. Antioxidants such as vitamins C and E were previously evaluated as an avenue to prevent EC dysfunction in preeclampsia. Vitamin C scavenges free radicals and Vitamin E prevents lipid peroxidation. However, the use of these vitamins for preeclampsia treatment has not been established yet.[23] Many studies has been exploring on natural products as the main source of antioxidants. It has been reported that celery herbs extract has an apigenin, flavonoid compound, which can prevent intrauterine growth restriction (IUGR) via suppression of antiangiogenic factor production in preeclampsia mice model. [24] Moreover, combination of nano herbal andaliman (*Zanthoxylum acanthopodium*) and EVOO affected the level of necrosis in hepatocyte cells on preeclampsia rats.[25]

The antioxidant properties of black rice bran in the present study might be associated with the presence of bioactive compounds found in black rice bran. Rice bran contains most of the biological components that include phenolic compounds, anthocyanins, phytic acid, γ - oryzanols, tocotrienols, and tocopherols, which have been previously reported as antioxidants[26-29]. The phenolic compounds in black rice bran is known to posses antioxidant activity[30,31].

CONCLUSION

Black rice bran reduced sFlt-1 levels, and increased PIGF in preeclampsia-induced HUVEC. These findings indicate anti-angiogenic properties of black rice bran (phenolic compounds) which can be a promising strategy in preeclampsia treatment. Further optimization and purification of black rice bran, are encouraged.

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