Scientific Research Publishing

Open Journal of Obstetrics and Gynecology, 2020, 10, 1686-1692
https://www.scirp.org/journal/ojog
ISSN Online: 2160-8806
ISSN Print: 2160-8792

The Role of Black Rice Bran (*Oryza sativa* L. "Sembada Hitam") on Levels of Malondialdehyde in Induction Human Umbilical Vein Endothelial Cell Serum Preeclampsia

David Randel Christanto[1*], Johanes C. Mose[1], Tety Yuniarti[2], Muhammad Begawan Bestari[3], Yekti Asih Purwestri[4], Prima Nanda Fauziah[5]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia
[2]Department of Pediatrics, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia
[3]Department of Internal Medicine, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia
[4]Biochemistry Laboratory, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia
[5]Department of Medical Medical Laboratory Technology, Faculty of Health, Universitas MH, Thamrin, Jakarta, Indonesia
Email: *david.randel@yahoo.com

How to cite this paper: Christanto, D.R., Mose, J.C., Yuniarti, T., Bestari, M.B., Purwestri, Y.A. and Fauziah, P.N. (2020) The Role of Black Rice Bran (*Oryza sativa* L. "Sembada Hitam") on Levels of Malondialdehyde in Induction Human Umbilical Vein Endothelial Cell Serum Preeclampsia. *Open Journal of Obstetrics and Gynecology*, **10**, 1686-1692.
https://doi.org/10.4236/ojog.2020.10120152

Received: December 1, 2019
Accepted: December 11, 2020
Published: December 14, 2020

Abstract

Preeclampsia is a multisystem disorder that contributes to morbidity and mortality worldwide. Recent studies show that elevated plasma and erythrocytic malondialdehyde (MDA), a marker of lipid peroxidation, has been documented in women with established preelampsia. This study aimed to observe antioxidant properties of black rice bran on preeclampsia. Level of MDA was measured with TBARS (*thiobarbituric acid-reactive substances*). Results showed that black rice bran has antioxidant properties by significantly decreasing MDA in preeclampsia-induced HUVEC. This makes black rice bran a promising agent which can be further used in preeclampsia treatment.

Keywords

Antioxidant, Black Rice Bran, MDA, Preeclampsia, TBARS

1. 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. According to SKDI (2012), preeclampsia/eclampsia contributes to about 13% maternal mortality in Indonesia [2]. Its incidences in Hasan Sadikin General Hospital (RSHS) were approximately from 4 to 10% in 2017, which contributes to 10.4% maternal mortality in the hospital [3].

Underlying mechanism of preeclampsia remains unclear. Many studies suggested that it involves genetic factor, immunologic factor, vascular disease, and imbalance between free oxygen radicals and scavengers in favour of oxidants. 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 [4]. Levels of plasma and erythrocytic malondialdehyde (MDA), a marker of lipid peroxidation in normotensive pregnant women, have been documented in women with established preelampsia [5] [6] [7]. Thus, antioxidants are considered as an 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 activities in agricultural waste. Black rice "Sembada Hitam" is a black rice cultivar in Yogyakarta, which is planted in Sleman and Bantul region, Indonesia [8]. Biological and antioxidant activity of rice is mainly localized in its bran (the outer layer) [9] [10].

Rice bran (the outer layer) is discarded during the milling process of white rice. However, the biological and antioxidant activity of rice is mainly localized in the rice bran [8] [11]. Rice bran contains most of the biological components that include phenolic compounds, anthocyanins, phytic acid, γ-oryzanols, tocotrienols, and tocopherols, which were previously reported as antioxidants [9] [10] [12] [13]. *Cempo Ireng* is a black rice cultivar in Yogyakarta, which is planted in Selman and Bantul region, Indonesia [14]. The phenolic compounds in black rice bran are reported to contain more than 1 phenolic hydroxyl group, which is assumed to poss antioxidant activity [15] [16]. 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 [11] [17]. This study aimed to observe anti-angiogenesis properties of black rice bran on preeclampsia through measurement of MDA levels.

2. Materials and Method

2.1. Study Design and Sampling

This was true experimental study with posttest only control group design. Re-

search 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.

2.2. Extraction of Black Rice Bran

Black rice bran was acquired from outer layer of black rice, and then filtered with 60 mm sieve. Black rice bran of 10 mg was macerated with 100 mL mixture of ethanol and HCl 1N (85:15) for 48 hours at room temperature. Extract was filtered with Whatman No.1 paper. Filtrate was remacerated with 50 mL mixture of ethanol and HCl 1N (85:15). Furthermore, extract was evaporated. Extract was stored in room temperature.

2.3. Cell Culture

HUVEC cell was cultured in RPMI 1640 containing 20% (v/v) sample (normal or preeclamptic serum) [18], 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 [19] [20] [21].

2.4. Lethal Concentration (LC_{50}) of Black Rice Bran toward HUVEC Cell

LC_{50} of black rice bran was measured with Brine Shrime Lethality Test (BSLT) [20] [21]. Cells (6×10^5 cell/mL) was added with black rice bran extract in various concentrations. Absorbance was read at 517 nm wavelength. LC_{50} was measured with the following formula:

$$\text{mortality percentage } \left(LC_{50}\right)\% = \frac{\left(A0 - A\right)}{A0} \times 100\%$$

A0 = Absorbance control; A = absorbance of the sample (HUVEC and various concentration of black rice bran extracts).

2.5. Measurement of MDA Levels

Cells (6×10^5 cell/mL) were placed into 96-wells microplate, and incubated at 37˚C 5% CO_2 (v/v). Futhermore, wells were washed with PBS 37˚C three or four times to discard remaining medium and unattached cells. Brand black rice in various concentration (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). Each well was washed with PBS pH 7.4 for 5 minutes, and cells were centrifuged at 3000 rpm 20 minutes. Supernatant was carried, and MDA was measured wtih TBARS (*thiobarbituric acid-reactive substances*) [22]. Supernatan was added with 15% w/v *trichloroacetic acid*, 0.375 w/v *thiobarbituric acid*, 0.25%

hydrichloric acid and 0.2% *triton* X. Afterward, cells were suspended using heating at 100˚C for 15 minutes and then centrifuged at 4500 rpm for10 minutes. Supernant was read at 532 nm wavelength.

2.6. Data Analysis

Data were analyzed with two way-ANOVA, and continued with post hoc Dunnett ($\alpha = 0.05$) using SPSS 21.

3. Results

LC_{50} of black rice bran is presented in Figure 1. Results showed LC_{50} of black rice bran was 500 ug/mL with mortality percentage 50.78%. This indicates LC_{50} less than 500 ug/mL shows non-toxicity towards HUVEC.

As shown in Table 1, MDA levels in preeclampsia-induced HUVEC were reduced after treatment of black rice bran ($p < 0.05$). At concentration of 62.5 µg/ml after 24 hours of incubation, MDA levels (5.0895 µM) were comparable to normal level in control group (5.7180 µM).

4. Discussion

In the present study, cytoxicity of black rice bran was determined through measurement of LC_{50} toward HUVEC cells (Figure 1). Results showed LC_{50} of black rice showed no toxicity toward HUVEC makes it appropriate for further treatment. Black rice bran exhibited very strong antioxidant properties in preeclampsia by decreasing MDA levels (Table 1). These results might be due to presence of bioactive compounds found in black rice bran. The phenolic compounds in black rice bran contain more than 1 phenolic hydroxyl group, which is known to poss antioxidant activity [15] [16]. Various phenolic acids are found such as ferulic acid, p-coumaric acid, vanillic acid, p-hydroxybenzoic, gallic acid, and protocatechuic acid [11] [17].

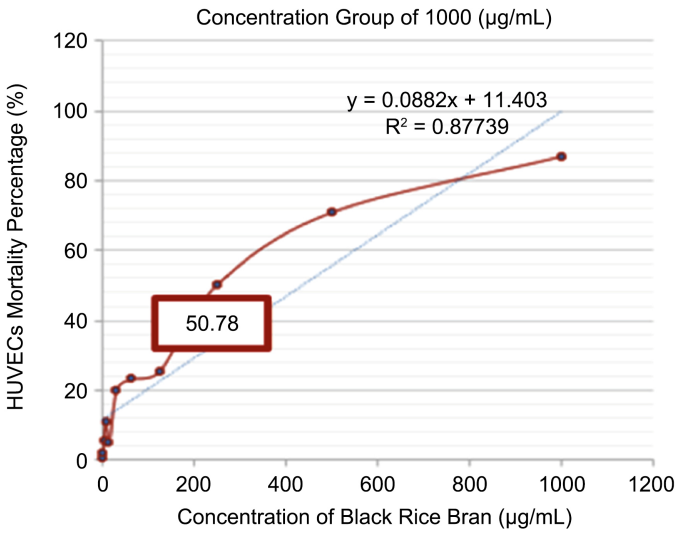

Figure 1. LC_{50} of black rice bran towards HUVEC.

Table 1. Level of MDA in preeclampsa-induced HUVEC treated with black rice bran.

Black rice bran (ug/mL)	Normal pregnancy		Preeclampsia		p-value
	24 hours incubation	48 hours incubation	24 hours incubation	48 hours incubation	
Control	5.7180 (0.0424)* (a)*	5.550 (0.0290) (b)	8.7085 (0.0078) (c)	8.8475 (0.0346) (d)	<0.001
1.953	4.2180 (0.0028) (a)	4.419 (0.0148) (b)	8.2730 (0.0509) (c)	8.3945 (0.0078) (d)	<0.001
3.906	4.8855 (0.0049) (a)	4.315 (0.0064) (b)	7.8880 (0.0014) (c)	7.7780 (0.0155) (d)	<0.001
7.8125	4.3815 (0.0714) (a)	4.187 (0.0198) (a)	7.2945 (0.0870) (b)	6.6655 (0.3118) (b)	<0.001
15.625	4.0140 (0.0071) (a)	4.070 (0.0573) (b)	6.5325 (0.0007) (c)	5.7625 (0.0007) (d)	<0.001
31.25	4.0090 (0.0028) (a)	3.941 (0.0071) (b)	5.7785 (0.0007) (c)	4.9925 (0.0007) (d)	<0.001
62.5	4.00 (0.00) (a)	3.896 (0.0141) (b)	5.0895 (0.0007) (c)	4.3250 (0.00) (d)	<0.001
125.0	3.9940 (0.0056) (a)	3.743 (0.0007) (b)	4.5775 (0.0007) (c)	3.6675 (0.0007) (d)	<0.001

Data are presented in mean. Different letters (a)-(c) in the same row indicates significant difference among tretament, analyzed with Duncan test ($p < 0.05$).

It has been reported that lipid peroxidation is the main causative factor for oxidative stress in preeclampsia. Free radicals initiate lipid peroxidation by attacking polyunsaturated fatty acids in cell membranes [23]. Uncontrolled peroxidation of fatty acids and cholesterol alter membrane fluidity and permeability as lipid peroxides are toxic compounds that damage endothelial cells, increase peripheral vasoconstriction and increase thromboxane synthesis and decrease prostacyclin synthesis [24]. Once steady state levels of blood lipid peroxides begin to rise, the stage would be set for self-perpetuating chain-reaction processes to take place. Endothelial contact with lipid peroxides would allow peroxidative damage of endothelial cell membrane lipids. Eventually, the permeability of the endothelium to plasma components, is reduced. Exposure of the vascular endothelium to lipid peroxides would begin to interfere prostacyclin synthesis, increasing the propensity for vasoconstriction and platelet aggregation [4].

5. Conclusion

Black rice bran has very strong antioxidant properties by significantly decreasing MDA levels in preeclampsia-induced HUVEC, which can be a promising strategy in preeclampsia treatment. Since this was a preliminary study to observe

efficacy of black rice bran which only through measurement of MDA levels, antioxidant activiy of black rice bran is not established yet. Further antioxidant measurements using other parameters, as well as *in vivo* study to determine optimum concentration of black rice bran, are encouraged.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Andrietti, S., Silva, M., Wright, A., Wright, D. and Nicolaides, K.H. (2016) Competing-Risks Model in Screening for Pre-Eclampsia by Maternal Factors and Biomarkers at 35-37 Weeks' Gestation. *Ultrasound in Obstetrics & Gynecology*, **48**, 72-79. https://doi.org/10.1002/uog.15812

[2] Badan Kependudukan dan Keluarga Berencana Nasional, Badan Pusat Statistik, Kementerian Kesehatan. (2013) Survei demografi dan kesehatan Indonesia (SDKI) 2012.

[3] Ginekologi, B.O. (2018) Data rekam medik dan laporan tahunan. Bagian Obstetri Ginekologi Fakultas Kedokteran Universitas Padjadjaran/RS Dr. Hasan Sadikin Bandung Tahun 2017. Bagian Obestetri dan Ginekologi RSHS, Bandung.

[4] Hubel, C., Roberts, J., Taylor, R., *et al.* (1989) Lipid Peroxidation in Pregnancy: New Perspectives on Preeclampsia. *American Journal of Obstetrics & Gynecology*, **161**, 1025-1034. https://doi.org/10.1016/0002-9378(89)90778-3

[5] Kabi, B.C., Goel, N., Rao, Y.N., Tripathy, R., Tempe, A. and Thakur, A.S. (1994) Levels of Erythrocytic Malondialdehyde, Vitamin E, Reduced Glutathione, G6PD Activity and Plasma Urate in Patient of Pregnancy Induced Hypertension. *Indian Journal of Medical Research*, **100**, 23-25.

[6] Henriksen, T. (2000) The Role of Lipid Oxidation and Oxidative Lipid Derivatives in the Development of Precelampsia. *Seminars in Perinatology*, **24**, 29-32. https://doi.org/10.1016/S0146-0005(00)80051-8

[7] Uotila, J., Tuimala, R., Pyykko, K. and Ahotupa, M. (1993) Pregnancy Induced Hypertension Associated with Changes in Maternal and Umbilical Blood Antioxidants. *Gynecologic and Obstetric Investigation*, **36**, 153-157. https://doi.org/10.1159/000292616

[8] Kong, S. and Lee, J. (2010) Antioxidants in Milling Fractions of Black Rice Cultivars. *Food Chemistry*, **120**, 278-281. https://doi.org/10.1016/j.foodchem.2009.09.089

[9] Iqbal, S., Bhanger, M.I. and Anwar, F. (2005) Antioxidant Properties and Components of Some Commercially Available Varieties of Rice Bran in Pakistan. *Food Chemistry*, **93**, 265-272. https://doi.org/10.1016/j.foodchem.2004.09.024

[10] Abdel-Aal, E.S.M., Young, J.C. and Rabalski, I. (2006) Anthocyanin Composition in Black, Blue, Pink, Purple, and Red Cereal Grains. *Journal of Agricultural and Food Chemistry*, **54**, 4696-4704. https://doi.org/10.1021/jf0606609

[11] Butsat, S. and Siriamornpun, S. (2010) Antioxidant Capacities and Phenolic Compounds of the Husk, Bran and Endosperm of Thai Rice. *Food Chemistry*, **119**, 606-613. https://doi.org/10.1016/j.foodchem.2009.07.001

[12] Schramm, R., Abadie, A., Hua, N., Xu, Z. and Lima, M. (2007) Fractionation of the

Rice Bran Layer and Quantification of Vitamin E, Oryzanol, Protein, and Rice Bran Saccharide. *Journal of Biological Engineering*, **1**, Article No. 9. https://doi.org/10.1186/1754-1611-1-9

[13] Moongngarm, A. and Saetung, N. (2010) Comparison of Chemical Compositions and Bioactive Compounds of Germinated Rough Rice and Brown Rice. *Food Chemistry*, **122**, 782-788. https://doi.org/10.1016/j.foodchem.2010.03.053

[14] BPTP Yogyakarta. Mari lestarikan beras hitam lokal 2010. [Diunduh tanggal 1 November 2017]. http://www.litbang.pertanian.go.id/artikel/238/pdf/Mengenal%20Beras%20Hitam%20dari%20Bantul.pdf

[15] Zhang, M.W., Zhang, R.F., Zhang, F.X. and Liu, R.H. (2010) Phenolic Profiles and Antioxidant Activity of Black Rice Bran of Different Commercially Available Varieties. *Journal of Agricultural and Food Chemistry*, **58**, 7580-7587. https://doi.org/10.1021/jf1007665

[16] Muntana, N. and Prasong, S. (2010) Study on Total Phenolic Contents and Their Antioxidant Activities of Thai White, Red and Black Rice Bran Extracts. *Pakistan Journal of Biological Sciences*, **13**, 170-174. https://doi.org/10.3923/pjbs.2010.170.174

[17] Laokuldilok, T., Shoemaker, C.F., Jongkaewwattana, S. and Tulyathan, V. (2011) Antioxidants and Antioxidant Activity of Several Pigmented Rice Brans. *Journal of Agricultural and Food Chemistry*, **59**, 193-199. https://doi.org/10.1021/jf103649q

[18] Neale, D., Demasio, K., Illuzi, J., Chaiworapongsa, T., Romero, R. and Mor, G. (2003) Maternal Serum of Women with Pre-Eclampsia Reduces Trophoblast Cell Viability: Evidence for an Increased Sensitivity to Fas-Mediated Apoptosis. *The Journal of Maternal-Fetal & Neonatal Medicine*, **13**, 39-44. https://doi.org/10.1080/jmf.13.1.39.44

[19] Bdolah, Y., Karumanchi, S.A. and Sachs, B.P. (2005) Recent Advances in Understanding of Preeclampsia. *Croatian Medical Journal*, **46**, 728-736.

[20] Gunardi, J.I., Mose, J.C., Satari, M.H., Anwar, A.D., Fauziah, P.N. and Riyuli (2016) Effects of Papua ant Nests (Myrmecodiapendens) on Level of sFlt-1, PIGF, MDA and NO in Preeclampsia-Induced HUVEC Cell Line. *International Journal of PharmTech Research*, **9**, 424-435.

[21] Basyir, V., Fauziah, P.N., Yanwirasti, Johannes, C.M. and Fadil, O. (2016) Effect of Lycopene on Level of Maondialdehid (MDA) in Preeclampsia-Induced Placental Trophoblast Cells. *International Journal of Pharmaceutical Research*, **10**, 103-108. https://doi.org/10.20902/IJPTR.2017.10115

[22] Janero, D.R. (1990) Malondialdehyde and Thiobarbituric Acid-Reactivity as Diagnostic Indices of Lipid Peroxidation and Peroxidative Tissue Injury. *Free Radical Biology and Medicine*, **9**, 515-540. https://doi.org/10.1016/0891-5849(90)90131-2

[23] Madazli, R., Benian, A., Gumustas, K., *et al.* (1999) Lipid Peroxidation and Antioxidants in Preeclampsia. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, **85**, 205-208. https://doi.org/10.1016/S0301-2115(99)00023-8

[24] Alexa, I.D. and Jerca, L. (1996) The Role of Oxidative Stress in the Etiology of Preeclampsia. *Revista medico-chirurgicala a Societatii de Medici si Naturalisti din Iasi*, **100**, 131-135.