

Ethanol Extract of Black Rice (*Oryza sativa* L) Bran Reduced Expression of RAGE in Skin of Wistar Rat Exposed with UVA and UVB

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Abstract: Aging is ongoing process and aging in skin is a most common problem which still undergoes research for aging prevention. In ageing there are 2 theories, first wear and tear theory and second programming theory. The first is including DNA damage, glycation, and free radical. Exposure of UV increase expression of AGE in skin followed by increasing of its receptor (RAGE) in fibroblast and followed by activation of NF-kB and increasing MMP and eventually collagen degradation which is hallmark of skin aging. Skin aging can be prevented by anti-oxidant like anthocyanin and black rice bran rich of anthocyanin with anti-inflammation and anti-glycation activity. Based on our review there is no data about effect of black rice bran from Jogjakarta with animal model of skin aging. Thirty-six Wistar rats (*Rattus norvegicus*) with 8-12 weeks old and 200-250 gr weight were randomly chosen and adapted for 7 days. Rats were divided into 2 groups and exposed with 20 mJ/cm² UVA and UVB for 4 weeks, five times a week. Control group only exposed to UVA and UVB and Treatment Group exposed to UVA and UVB with orally 300 mg/kg weight extract. After 4 weeks the rats were sacrificed and RAGE expression in skin was determined by immunohistochemistry. Independent T-test analysis found significant difference in percentage of fibroblast that expressed RAGE ($p < 0.05$). The control group expressed RAGE for 51.396% and the treatment group expressed RAGE for 15.275%. There was decrease of RAGE expression for 35.671% between control and treatment group. This study showed that ethanol extract of black rice bran reduced expression of RAGE in fibroblast of skin Wistar rat exposed with UVA and UVB significantly.

Keywords: black rice bran, glycation, aging, RAGE

1. Introduction

Aging is ongoing process in all living organism and miraculously ageing can be prevented, treated and reverted back to optimal youth [1,2]. In aging there are 2 theories, first wear and tear theory and second programming theory. The first is including DNA damage, glycation, and free radical [3]. Glycation is advancing so fast since the discovery its involvement in diabetic pathogenesis [4]. Aging in skin is a most common problem which still undergoes research for aging prevention. Risk factors for skin aging are sunlight exposure, smoking, diet, infection, hormonal disruption, reactive oxygen species (ROS), reactive carbonyl species (RCS), and Advanced Glycation End-products (AGEs) [1-6]. These factors will induce NF-kB and eventually will increase production of matrix metalloproteinase (MMPs) which will decrease the collagen in skin layer [6-13]. Exposure of ultraviolet (UV) also increase expression of AGE in skin followed by increasing of its receptor (RAGE) in fibroblast and endothelium. Eventually RAGE activates NF-kB followed by increasing production of MMP and MMP degrades collagen fiber in dermis [13-22].

Aging can be prevented by antioxidant and of its kind is anthocyanin [23]. Anthocyanin rich in nature especially in plant [24]. Black rice (*Oryza sativa* L) bran is rich with anthocyanin and its purpose is well known for anti-inflammation and anti-glycation. Anthocyanin can prevent activation of NF-kB signaling in some studies [23-28]. However, there is no study about effect of black rice bran in

animal model of skin aging especially for RAGE expression and this research will reveal effect of ethanol extract of black rice bran from Sleman, Jogjakarta, Indonesia.

2. Material and Methods

2.1 Black Rice Bran Extract

Black rice bran was obtained from Sleman, Jogjakarta, Indonesia. As much of 1.5 kg of black rice bran was mixed with 70% ethanol in room temperature, then homogenized for 3 hours. Maceration for 24 hours at 4°C and filtered through Whatman Paper no. 1. Then the filtrate was evaporated in Rotary Evaporator then the extract was kept at 4°C and protected from direct sunlight.



Figure 1: Black Rice (*Oryza sativa* L)

2.2 Animals

Thirty-six Wistar rats (*Rattus norvegicus*) were obtained from UPT. Laboratorium Analitik, Udayana University. The Ethical Clearance of this study was no: 335a/KE-PH-Lit-III/XII/2017. Rats with 8-12 weeks old and 200-250 gr weight were randomly chosen and adapted for 7 days. Rats were divided into 2 groups and exposed with 20 mJ/cm² UVA and UVB for 4 weeks, five times a week. First group (Control) was rats with exposure of 20 mJ/cm² UVA and UVB for 4 weeks, five times a week. Second group (Treatment) was rats with exposure of 20 mJ/cm² UVA and UVB for 4 weeks, five times a week and orally 300 mg/kg weight extract of black rice bran. After 48 hours from the last exposure, the rats were euthanized by using injection of Ketamine and Xylazine.

2.3 RAGE Examination

Briefly, after euthanized, the biopsy of skin was immersed into formalin buffer for a night. The skin dehydrated by using grading ethanol, clearing and embedding into paraffin at 60°C. The thickness of cutting was 5 µm and then placed on object glass with poly-lysine. Antigen retrieval was using TE pH 9 boiled with 700-watt microwave for 15 minutes followed 240-watt for 5 minutes, the cooled in room temperature. Next, the skin washed 2 times in PBS, then peroxidase blocking for 10 minutes, incubation with labeled polymer-HRP for 30 minutes, and then DAB mixture for 10 minutes. Then counterstain with Meyer Hematoxylin then mounting with Entellan and cover glass. Photomicrograph was using 400x magnification by microscope CX-41 (Olympus, Japan) and camera OptilabPro (Miconos, Indonesia). Picture of skin was obtained and analyzed to count the percentage of fibroblast cell which expressed RAGE. Each sample was captured 3 fields for 400x magnification.

2.4 Statistic

The data obtained then analyzed with independent T-Test by using SPSS 16.0 software.

3. Results

Independent T-test analysis found significant difference in percentage of fibroblast that expressed RAGE ($p < 0.05$) (Figure 2). The control group expressed RAGE for 51.396% and the treatment group expressed RAGE for 15.275%. There was decrease of RAGE expression for 35.671% between control and treatment group. This study showed that ethanol extract of black rice bran reduced expression of RAGE in fibroblast of skin Wistar rat exposed with UVA and UVB significantly ($p=0.000$). Figure 3 and 4 showed percentage of fibroblast which expressed RAGE in Control and Treatment group respectively.

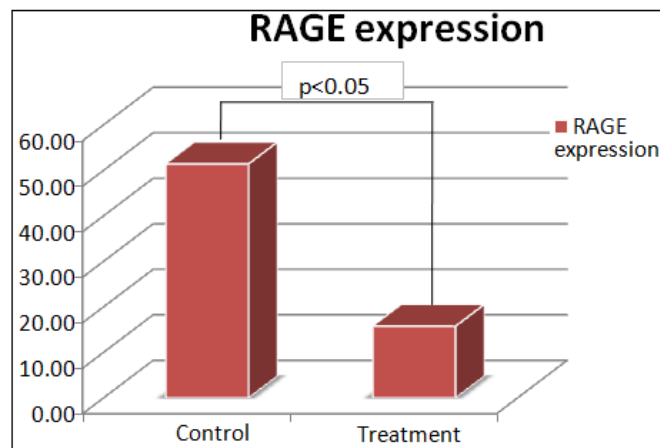


Figure 2: Expression of RAGE between Control and Treatment Group.

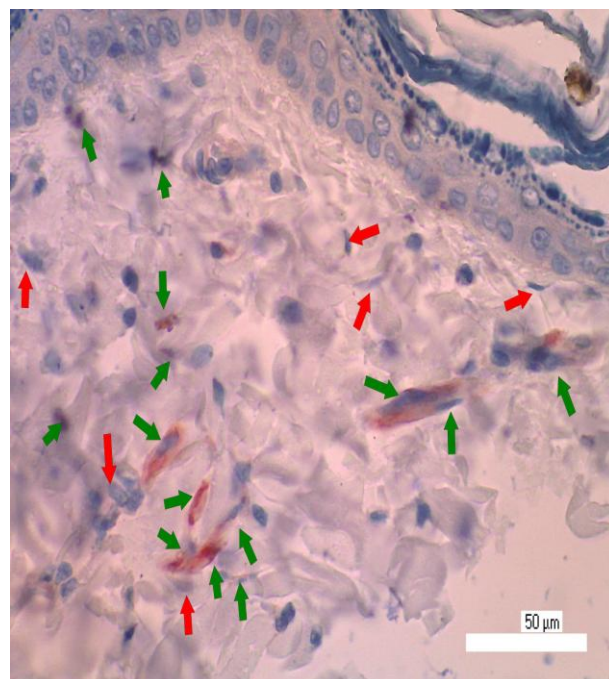


Figure 3: Expression of RAGE in Control Group. Fibroblast which expressed RAGE (green arrow). Fibroblast which was not expressed RAGE was red arrow (400x magnification).

4. Discussion

This research revealed antioxidant properties of black rice bran which contains Black rice bran is rich with anthocyanin and its purpose is well known for anti-inflammation and anti-glycation [23, 25, 26]. Anthocyanin can prevent activation of NF-κB signaling in some studies thus will decrease MMP production and the end, degradation of collagen will be prevented [6-13]. This study showed that expression of RAGE in fibroblast significantly reduced by 35.671% between control and treatment group (51.396% vs 15.275%, $p = 0.000$). Reduced RAGE expression in fibroblast probably through anti-oxidant and anti-glycation properties of anthocyanin [23-28].

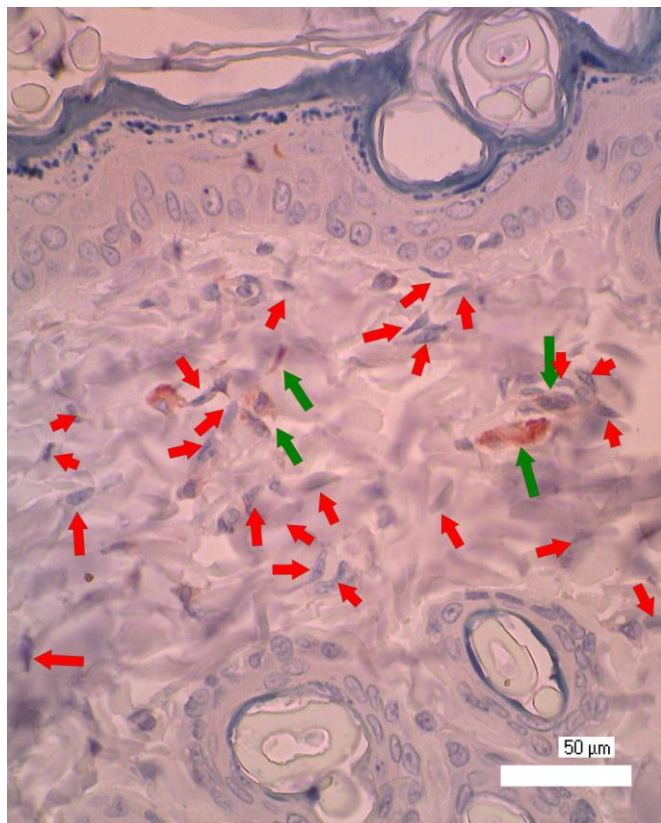


Figure 4: Expression of RAGE in Treatment Group. Less of fibroblast that expressed RAGE (green arrow) compared to Control Group (Figure 3). Fibroblast which was not expressed RAGE was red arrow (400X magnification)

5. Conclusion

Black rice bran ethanol extracts effectively reduced expression of RAGE in skin fibroblast of rat exposed with UVA and UVB through its antioxidant and anti-glycation properties.

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