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# Black rice bran (*oryza sativa l. indica*) extract cream prevented the increase of dermal matrix metalloproteinase-1 and dermal collagen reduction of male Wistar rats (*rattus norvegicus*) exposed to ultraviolet-B rays

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**Introduction:** The radiation of UV B rays exposure is capable of inducting free radicals to the skin, damaging the dermal collagen and stimulating skin aging. Photoaging prevention may be conducted by utilizing antioxidant and one of the good sources of antioxidant is black rice bran. Therefore the purpose of this research was to assess the effects of topical 35% black rice bran (*Oryza sativa L.indica)* extract toward dermal Matrix Metalloproteinase-1 (MMP-1) and dermal collagen in male Wistar rats (*Rattus norvegicus*).

**Method:** A post-test only control group design was conducted using 36 male Wistar rats divided into 2 groups (treatment and control). Both groups were exposed to UV-B rays 3 times a week for 4 weeks but the treatment group received 35% black rice bran extract cream twice a day. The control group was only treated by base cream. Histopathological examination was used to assess MMP-1 expression while Picro Sirius Red staining was used to assess dermal collagen on the rat's skin.

**Result:** Comparative statistical analysis results for both groups revealed that MMP-1 expression in treatment group was significantly lower than control group (30.94±4.22% vs. 9.57±2.76%; $p<0.001$). Meanwhile, collagen density was significantly higher in treatment group (83.59±2.84% vs. 58.40±2.69%).

**Conclusion:** Topical application of 35% black rice bran extract cream prevented the increase of MMP-1 expression and dermal collagen reduction. Further study is needed to validate these findings.

**Keywords**: black rice bran extracts cream, MMP-1 expression, dermal collagen, ultraviolet-B rays

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## INTRODUCTION

One of the external factors that triggered aging process is environmental factors such as ultraviolet radiation (UV) from sunlight. Radiation by UV rays is divided into 3 categories namely UVA, UVB, and UVC radiations.[1] Human skin is continuously exposed to air, solar radiation, environmental pollution and chemical substances contamination capable of inducing free radicals formation such as *Reactive Oxygen* Species (ROS) which results in oxidative stress and stimulates skin aging. UV ray radiation was the main factor of skin aging. Skin aging was marked by changes in the dermal connective tissue such as collagen and elastin degradation, clinically manifesting as wrinkles.[2] Collagen is the main component of connective tissue and main extra cellular protein of human body which protect organs from external trauma and provided structural integrity and stability to the dermis. Around 80% of the human skins' dry weight comprised of collagen.[3]

Direct or indirect UVB radiation initiates and activates a complex process of biochemical reaction to the skin. On molecular level, radiation by UVB ray activates the surface receptor of keratinocytes and fibroblast cell in the skin and induce signal transduction cascade.[4] And then various molecular changes occurs such as transcription factor activation like protein-1 (AP-1) activation which regulates Matrix Metalloproteinases (MMPs) expression especially Collagenase-1 (MMP-1) which degrade dermal collagen and inhibit collagen deposition.[5,6]

Photoaging prevention can be achieved by consuming antioxidants which are substances that slows, prevents, or eliminates molecular damages due to oxidation reaction.[5] Skin has a network of protective antioxidants such as endogenous enzymatic antioxidants like Glutathione peroxidase, Superoxide dismutase (SOD), and catalase, and non-enzymatic antioxidant with low molecular weight like glutathione (GSH),uric acid and ubiquinol. However, extreme UV radiation is capable of diminishing and even completely depleting skin's antioxidant reserve.[10]

Black rice bran is part of the inner skin layer in the black rice hull from black rice plant. Indonesia is renowned as an agrarian country with a majority of the people working in farming industry and also third largest global rice producer.[11] In the rice milling process, the husk shall be separated to obtain brown rice. The next step is the double threshing phase, in which the first threshing produces chaff (rough fiber), while the second threshing produces rice bran.[12] As side effect from rice threshing, rice bran contains good nutrient contents and rich in antioxidants. The antioxidant substances contained in the rice bran consists of phenolic acid, flavonoid, anthocyanin, proanthocyanidins, tocopherol, tocotrienols, *γ-oryzanol* and phytate acid.[13]

Therefore, this research was conducted to test the administration of black rice bran extract toward MMP-1 expression and dermal collagen level in the skin of male Wistar rats exposed to UVB rays.

## METHODS

**Study Design and Sample:** A post-test only control group study was conducted at the Division of Drug and Animal Development Division of the Department of Pharmacology and Therapy, Faculty of Medicine, Udayana University. The samples of this research were 36 male Wistar rats (Rattus norvegicus), aged 6-8 weeks old, and ± 150 – 200 gram in weight. All rats from each group had had their back fur removed for cream administration. Rats were divided into 2 groups: control group (18 rats) were exposed to UV B rays and received basic cream while treatment group (18 subjects) were exposed to UV B rays and treated with 35% black rice bran extract cream.

**Cream Production:** 35% black rice bran extract cream was made from black rice bran extract from the rice planted in Gasol Village, Cianjur, West Java and extracted using 96%: HCl (9:1), 1 part bran: 10 part solvent to be macerated and filtered to obtain filtrate. The filtrate was steamed using rotary evaporator on 40°C, 50 mBar vacuum pressures, and 100 rpm in angular speed until crude extract was obtained to be analyzed by phytochemical analysis test. The extract was mixed with base cream to obtain homogenous form. Base creamused in this study was composed of 15% Strearic Acid, 2% Triethanolamine (TEA), 25% liquid Paraffin, 0.18% Nipagin, 0.2% Nipasol and 100% aquadest.

**UV Exposure:** UVB ray exposure was conducted at a frequency of 3 times a week (Monday, Wednesday and Friday) started with 50 mJ/cm$^2$ for 50 seconds during the first week, followed by 70 mJ/cm$^2$ for 70 seconds during the second week and 80 mJ/cm$^2$ for 80 seconds in the last two weeks with the total UVB received at 840 mJ/cm$^2$. Radiation was conducted every day at 10.00 Am using Phillips UVB PL-S9W/01/2P lamp.

**Cream administration:** Cream was applied at 0.1 ml/cm$^2$ on the back skin using 2 cc syringes to collect base cream and 35% black rice bran extract cream and applied evenly using cotton buds. At the time of UVB ray exposure, the cream was administrated 2 times a day for 4 weeks, 20 minutes after UVB ray exposure on 09.40 AM local time, 10.00 AM and 4 hours later the cream administration is restarted on 14.00 PM.

At the end of the study, a skin biopsy (2 cm x 2 cm x 2 mm) was taken and then assessed using immunohistochemistry staining to calculate the MMP-1 expression and Picro Sirius Red staining for dermal collagen density.

## RESULTS

Biopsy samples were taken from all rats and assessed histologically. The result of MMP-1 immunohistochemistry and Sirius red are depicted in figure 1. It clearly shows the difference between control and treatment group. The expression of

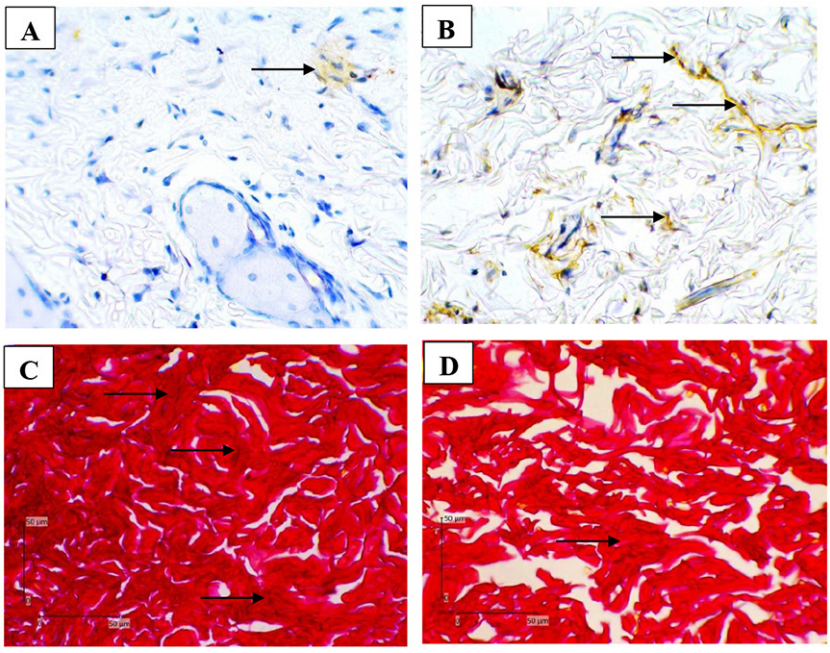


**Figure 1.** MMP-1 expression was strikingly lower in treatment group (A) compared to control group (B). Meanwhile, the density of dermal collagen was lower in control group (D) compared to treatment group (C). The samples were observed under light microscope at 400X magnification

**Table 1. Comparison MMP-1 Expression and Amount of Dermal Collagen Between Groups**

| Variable | Group | | Mean Difference | 95% CI | P Value |
|---|---|---|---|---|---|
| | Control (n=18) | Treatment (n=18) | | | |
| MMP-1 Expression | 30.94±4.22 | 9.57±2.76 | -21.37 | -23.79-(-18.95) | <0.001 |
| Dermal Collagen Level | 83.59±2.84 | 58.40±2.69 | 25.19 | 23.32-27.07 | <0.001 |

MMP-1 is strikingly lower in treatment group (A) compared to control group (B). Meanwhile, the density of dermal collagen was also higher in treatment group (C) compared to control group (D).

The comparison data between treatment and control group was conducted using independent t-test to analyze mean difference between the groups. The analysis showed that the mean of MMP-1 expression in control group was significantly higher at 30.94±4.22 compared to the control group (9.57±2.76) (p<0.001). In contrast, the density of dermal collagen was seemed to be significantly higher in treatment group (83.59±2.84) compared to control group (58.40±2.69) (p<0.001) (Table 1).

## DISCUSSION

In this study, we found that 35% black rice bran extract cream was able to repress MMP-1 expression by fibroblast and increase the density of dermal collagen. This experiment was never published before and this is the first evidence of black rice bran extract as inhibitor of photo-aging. However, the link between MMP-1 suppression and increased collagen density is still not strong enough to explain the effect of black rice bran extract on both variables since it is not yet clear whether the black rice bran extract directly or indirectly inhibit the expression of MMP-1 and whether it also increase collagen synthesis or just simply inhibit collagen degradation by suppressing MMP-1.

Regarding its content, black rice bran is known to contain high amount of anthocyanin. Anthocyanin is known to prevent collagen damage due to UV light exposure when applied topically. It also prevents the reduction in pro-collagen expression, enhancing collagen transcription, and inhibits MMP-1 expression through suppression nuclear factor- κappa beta (NF-κB). The other mechanisms include inhibition of c-jun phosphorilation, p53 activation, and activation of activator of transcription-1 (STAT-1) related signaling which is related to MAPK signaling.[14]

## CONCLUSION

Topical administration of 35% black rice bran extract cream prevent increased expression of MMP-1 and enhance dermal collagen density in the skin of male Wistar rats exposed with UV B rays.

## CONFLICT OF INTEREST

All authors declared that there is no conflict of interest regarding this publication

## AUTHOR CONTRIBUTION

All authors contributed equally in the writing of this article

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This study was self-funded without any contribution from third party.

## ETHIC APPROVAL

This study had been ethically approved by ethical commission of Faculty of Medicine Udayana University with approval letter number 409/KE-PH-Lit-2/VII/2019

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