Administration of 15% Red Beet (Beta Vulgaris) Extract Cream Inhibited The Increased Expression of Mmp-1 and The Decreased Amount of Collagen in Wistar Strained Rat (Rattus Norvegicus) Exposed to Ultra Violet-B Light

I Gusti Bagus Ketut Oka Parwata, I Made Winarsa Ruma, and Ni Wayan Winarti

ABSTRACT

Aging is a natural thing that happens to all individuals. Currently, the theory of the causes of aging is starting to develop, with this as the basis for efforts to prevent aging to achieve longevity in individuals in good health and quality. This occurs due to exposure to UVB rays exceeding the dose and repeatedly for a long time. This also results in collagen degradation and an even higher increase in MMP-1. Betalain pigment, which gives red beetroot its reddish-purple color, is the most important component. Beet tubers in several studies are the ten fruits that have the greatest concentration of antioxidants.

This research is an experimental study using a post-test-only control group design. Subjects were 30 Wistar rats, divided into 3 treatment groups where each group consisted of 10 rats. Measurement of MMP-1 using the immunohistochemical method and size of collagen with Pico-Sirius-Red staining.

The group of mice with UVB exposure intervention and given beetroot cream had the lowest average MMP1 expression, 25.11%, and had a higher average amount of collagen, 80.08% pixels. There was a significant difference in the mean MMP-1 expression and the amount of collagen in the three observation groups. The correlation test showed that the correlation coefficient (r) between the administration of red beet root extract (Beta vulgaris) inhibited the increase in MMP-1 expression with a strong relationship (r = -0.712). Meanwhile, the administration of red beet root extract (Beta vulgaris) inhibited the decrease in the amount of collagen with a strong association strength (r = 0.785).

Topical administration of red beetroot (Beta vulgaris) 15% extract cream can inhibit the increase in MMP 1 expression and decrease in collagen in the skin of Wistar rats exposed to ultraviolet B light as seen from the mean difference and strong correlation strength.

Keywords: Collagen, MMP-1, Red beetroot, UVB.

I. INTRODUCTION

Aging is a natural thing that happens to all individuals. The mechanism of aging occurs gradually in all parts of the body and organs. There are two types of aging, namely chronological aging, and biological aging. Chronological aging, it includes aging that cannot be inhibited, in this case, it is like getting older. While biological aging includes aging that can be inhibited through disease prevention or the aging mechanism itself (Pangkalahia, 2007).

Currently, the theory of the causes of aging is starting to develop, with this as the basis for efforts to prevent aging to achieve longevity in individuals in good health and quality. This makes humans try not to grow old with various therapeutic efforts. However, this therapy still needs further research because this knowledge is still new.

The causes of aging are divided into two factors, namely internal and external factors. The internal factors consist of free radicals, hormonal changes, methylation, glycosylation cell damage, and decreased immune conditions. While external factors consist of pollution, stress levels, economy, and lifestyle (Pangkalahia, 2011). In particular, aging of the skin occurs due to external factors such as the consumption of alcohol and cigarettes, nutritional intake, and exposure to ultraviolet (UV) rays. Repeated exposure to UV light can cause photodamage. Photodamage often occurs on parts of the skin that are directly exposed to the sun, for example, the face, arms, and chest. Symptoms that can arise as a result of...
photoaging can include skin experiencing wrinkles, roughness, pigment lesions, and up to malignancy (Geng et al., 2021).

Free radicals in cells or called reactive oxygen species (ROS) are caused by cell cross-linking damage to pyrimidine bases. This occurs due to exposure to UVB rays exceeding the dose and repeatedly for a long time. This also results in higher collagen degradation. The mechanism for increased collagen degradation started with exposure to UVB rays which resulted in the formation of ROS which in turn induced matrix metalloproteinases (MMPs) (Alam & Havey, 2010; J Krutmann, 2011).

The process of collagen damage due to photoaging begins with collagen undergoing glycation. Glycation is a non-enzymatic reaction, in which sugar reduces the extracellular matrix molecules of collagen and protein. As a result of this, collagen loses its flexibility so it cannot return to its original state. In addition to experiencing glycation, collagen also undergoes degradation and inhibits the growth of procollagen (Geng et al., 2021).

One of the deterrents and inhibitors of this process is antioxidants. Independently the skin already contains antioxidants such as superoxide dismutase (SOD), catalase, glutathione peroxidase, tocopherol (vitamin E), coenzyme Q10 (CoQ10), ascorbic acid (vitamin C) and carotenoids, but the amount contained is not yet effective in overcoming oxidative stress. experienced.

The mechanism for protecting collagen from the effects of UV rays by antioxidants is by neutralizing free radicals in the skin through electron donation. Meanwhile, the anthocyanin mechanism prevents collagen damage by preventing the degradation of mature collagen and the reduction of new collagen due to transcription factor (Apak et al., 2007; Telang, 2013).

Red beetroot is a source of anthocyanins and high levels of phenolic compounds. A plant such as red beetroot (Beta vulgaris). Betalain pigment, which gives red beetroot its reddish-purple color, is the most important component. Beet tubers in several studies are the ten fruits that have the greatest concentration of antioxidants. There are two subclasses of betalain, namely betacyanin (violet-red) and betaxanthin (orange-yellow). The concentration of antioxidants in red beetroot is 1.98 mmol/100 g, making it one of the most antioxidant-rich foods. Flavonoids (350-2760 mg/kg), betacyanins (840-900 mg/kg), betanins (300-600 mg/kg), ascorbic acid (50-868 mg/kg), and carotenoids (0.44 mg/kg) constitutes the majority of the antioxidant composition of red beets (Ananda, 2008). Red beets also include tubers that are easy to obtain in Indonesian traditional markets (Nanda, 2014).

To find out whether red beetroot extract can suppress MMP 1 expression and inhibit the decrease in collagen levels in the skin of Wistar rats exposed to ultraviolet B radiation, researchers wanted to test this hypothesis using red beetroot extract (Beta vulgaris). The results of this study are expected to be a reference in efforts to prevent and treat new skin aging in the future.

II. MATERIAL AND METHODS

A. Methods

This research is an experimental study using a post-test only control group design. The research was carried out from August 2022 to December 2022 at the Histology Laboratory, Faculty of Medicine, Udayana University. Red beetroot extract is made at the Laboratory Service Unit of the Faculty of Agricultural Technology, Udayana University. The preparation of beetroot extract cream and base cream was made at the Pharmacology Laboratory of the Faculty of Medicine, Udayana University. Subjects were 30 Wistar rats, divided into 3 treatment groups where each group consisted of 10 rats. Group P0 was without treatment, group P1 was treated with ultraviolet B exposure and basic cream, and group P2 was treated with ultraviolet B exposure and 15% red beetroot (Beta vulgaris) extract cream. MMP-1 measurement using immunohistochemistry method and collagen measurement with Pico-Sirius-Red staining.

B. Data Analysis

Statistical analysis was performed using Statistical Package for Social Science (SPSS) software for Windows version 24. All data were tested for normality using Shapiro-Wilk. The significance test used One-Way Annova (parametric test) with Post Hoc Least Significant Difference. Meanwhile, the non-significant data used the Kruskal-Wallis test with the Mann-Whitney Post Hoc.

III. RESULTS

A. Administration of Red Beetroot (Beta vulgaris) Extract Cream Against MMP-1 Expression in Wistar Strain Mice Exposed to Ultra Violet-B Light

MMP 1 expression was observed using immunohistochemical staining. After immunohistochemical staining, the three treatment group mice were photographed using an LC evolution camera and an Olympus microscope with an objective magnification of 400 times to observe MMP 1 expression. The following is a picture of the dermis tissue of the test animals that had been carried out by immunohistochemistry.

![Fig 1. MMP 1 Expression in Dermis Tissue by Immunohistochemical Stainin](image-url)
Based on the results of observations, it can be seen that the expression of MMP-1 in the P2 group was lower than the P0 and P1 groups as shown by the red arrows. The results of calculating MMP1 expression in the three groups of mice by calculating the percentage of fibroblasts expressing MMP 1 from all tissue areas showed that the group of mice with UVB exposure intervention and given beetroot cream had the lowest average MMP1 expression, which was 25.11% compared to the two observation groups others (Table I).

<table>
<thead>
<tr>
<th>Observation Group</th>
<th>Mean (% cell)</th>
</tr>
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<tbody>
<tr>
<td>P0</td>
<td>36.94 ± 5.15</td>
</tr>
<tr>
<td>P1</td>
<td>55.76 ± 3.96</td>
</tr>
<tr>
<td>P2</td>
<td>30.54 ± 7.42</td>
</tr>
</tbody>
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Shapiro-Wilk was used for the normality test. MMP-1 expression MMP-1 expression data in the P2 observation group showed that the data were not normally distributed with a p value <0.05. Statistically using the Kruskal-Wallis test it can be concluded that there is a significant difference in the mean MMP-1 expression between groups (p <0.05). Then a comparison test was carried out between treatment groups with the Post Hoc Mann-Whitney presented in the Fig. 2.

The results of the Mann-Whitney Post hoc test showed that the p-value <0.05 (Fig. 2). This means that all observation groups have a significant difference in mean compared to other groups. It can be seen that the mean value of MMP-1 expression in the group of mice with UVB exposure intervention and given red beetroot cream was smaller than the group without treatment and the group with UVB exposure intervention and given essential cream.

Based on the calculation of these results, it can be interpreted that the treatment of giving red beetroot cream to rats (Rattus Norvegicus) Wistar strain exposed to UV-B light had a significant effect on inhibiting the increase in MMP-1 expression.

B. Administration of Red Beetroot (Beta vulgaris) Extract Cream Against MMP-1 Expression in Wistar Strain Mice Exposed to Ultra Violet-B Light

Observation of the amount of collagen was carried out using Sirius red staining. After Sirius red staining, the three observation groups were photographed using an LC evolution camera and an Olympus prepare microscope for each preparation to observe the amount of collagen. The following is a picture of the skin tissue on the back of the test animal stained with Sirius Red.

Based on the results of observations, it can be seen that the P2 observation group (the group exposed to UVB and given beetroot cream) in Fig. 3C shows collagen expression with red collagen fibers appearing wider and thicker. The results of these observations can be calculated in the amount of collagen in the three groups of mice by calculating the percentage of collagen from all areas of tissue that appear bright red. The results of the calculation of the group of mice with UVB exposure intervention and given beetroot cream had an average amount of collagen that was higher than the other two observation groups, which was 80.08% pixels (Table II).

<table>
<thead>
<tr>
<th>Observation Group</th>
<th>Mean (% pixel)</th>
</tr>
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<tbody>
<tr>
<td>P0</td>
<td>74.99 ± 1.47</td>
</tr>
<tr>
<td>P1</td>
<td>69.38 ± 0.89</td>
</tr>
<tr>
<td>P2</td>
<td>80.08 ± 0.88</td>
</tr>
</tbody>
</table>

Shapiro-Wilk was used for the normality test. The amount of collagen in all study groups showed a normal data distribution (p > 0.05). Statistically using the One-Way ANOVA test it can be concluded that there is a significant difference in the mean amount of collagen between groups (p <0.05). Then a comparison test was carried out between treatment groups with the Post Hoc Least Significant Difference presented in the following Fig. 4.

Based on the results of the Post Hoc Least Significant Difference test, it showed that the p value <0.05 (Fig. 4). This means that all observation groups have a significant difference in mean compared to other groups. This means that all observation groups have a significant difference in mean compared to other groups. It can be seen that the mean value of collagen in the group of mice with UVB exposure intervention and given red beetroot cream was greater than the group without treatment and the group with UVB exposure intervention and given basic cream. Where the mean difference between the P2 observation group (the group exposed to UVB and given red beetroot cream) and the P0 observation group (the group without treatment) was 10.70
and the P1 observation group (the group exposed to UVB and given basic cream) was 5.09.

Based on the calculation of these results, it can be interpreted that the treatment of giving red beetroot cream to rats (Rattus Norvegicus) Wistar strain exposed to UV-B light had a significant effect on inhibiting the decrease in the amount of collagen.

![Graph showing comparison of collagen expression between groups](image)

**Fig 4. Comparison test results of group means for observation total collagen in Wistar strain rats (Rattus Norvegicus) exposed to Ultra Violet-B Light.**

C. Administration of Red Beetroot (Beta vulgaris) Extract Cream Against MMP-1 Expression in Wistar Strain Mice Exposed to Ultra Violet-B Light

Observation of the amount of collagen was carried out using Sirius red staining. After Sirius red staining, the three observation groups were photographed using an LC evolution camera and an Olympus prepare microscope for each preparation to observe the amount of collagen. The following is a picture of the skin tissue on the back of the test animal stained with Sirius Red.

While the correlation coefficient (r) between administration of beetroot extract (Beta vulgaris) and the amount of collagen is 0.785, which means that the direction of the correlation between the two variables is positive. This means that the administration of red beetroot extract (Beta vulgaris) inhibits the decrease in the amount of collagen with a strong bond strength.

IV. DISCUSSION

Based on the results of univariate analysis, it can be seen that the group of mice treated with UVB exposure and given beetroot cream had the lowest average MMP1 expression, ie 25.11% compared to the other two observation groups. While the highest average was 55.76% in the UVB-exposed group and given basic cream. This was evident from the observation that the dermis tissue of the group exposed to UVB and given red beetroot cream showed less MMP-1 expression (brown color) than the group with base cream.

This is in line with research conducted by Jung et al (2021), where UV-B light affects increased expression of MMP-1. This is also supported by other studies, where MMP-1 is secreted by keratinocytes and dermal fibroblasts in response to UV radiation stimulation (Pittayapruk et al., 2016).

Repeated exposure to UV radiation accelerates premature skin aging or photoaging (Todorova and Mandinova A, 2020). Skin wrinkles are mainly caused by UV rays which increase the expression of MMP-1 protein and oxidative stress and deplete collagen in the skin (Kim and Lee, 2018). UV-induced regulation of MMP-1 has a direct impact on the skin wrinkling process (Roh et al., 2017). Overexpression of MMP-1 by UV irradiation facilitates skin wrinkling by disrupting tissue integrity. Previous studies have shown that UV-activated MAP kinase pathways, including JNK and p38, lead to AP-1 activation, which further increases MMP-1 expression. The SUV activates the MAPK and Akt lines. Notably, UV radiation can activate the ERK signaling pathway, which plays a critical role in the activation of the AP-1 transcription factor. AP-1 activity is enhanced by the MAPK and Akt pathways through c-Fos and c-Jun phosphorylation, because AP-1 consists of a c-Jun homodimer or c-Jun and c-Fos heterodimers. In epidermal and dermal cells, AP-1 forms a complex to regulate the transcription of MMP-1, which is an important transcription factor. MMP-1 mRNA upregulation is modulated by AP-1. Photaging which is characterized by skin wrinkling is an aging process that occurs naturally. Thus, it is important to find new agents that can inhibit or delay the photaging process (Kim and Lee, 2018; Li et al., 2018).

Based on the results of the mean difference analysis using the Kruskal-Wallis test, a significant difference was obtained in the mean MMP-1 expression between untreated rats, treatment group P1 (Group exposed to UVB and given basic cream), and P2 (Group exposed to UVB and given red beetroot cream). In addition, the correlation test analysis obtained a p value of 0.000 (p < 0.05) and a correlation coefficient (r) of -0.712, which means that the direction of the correlation between the two variables is negative. This means that red beetroot extract (Beta vulgaris) inhibits MMP-1 expression with a strong association strength. This means that the administration of red beetroot cream to the Wistar strain (Rattus Norvegicus) rats exposed to UV-B light had a significant effect on inhibiting MMP-1 expression.

Red beets contain a number of bioactive compounds that may exhibit health-promoting effects, including betalains, ascorbic acid, flavonoids, carotenoids, polyphenols, saponins and high levels of nitrates (Clifford et al., 2015). Betalains have uniform structural characteristics derived from betalamic acids, together with the R1 or R2 radicals, where the substituents can be either hydrogen or radicals. Variations in the substituent group stem from the different origins of the pigments and affect their stability and pattern. According to the chemical structure, it is relatively easy to extract betalain. Two predominate forms include yellow betaxanthins and redviolet betacyanins. Beets have around 75–95% betacyanin and 5–25% betaxanthin (Sitompul and Puspita Zulfati, 2019). More than 80% of the pigment in red beetroot is composed of betacyanins, namely betanin and isobetanin, isomers of betanin. A previous study investigated the betain content in red beetroot juice and reported that betainin served as the most abundant component (300–600 mg/kg), followed by vulgaxanthin and isobetainan (Slavov et al., 2013). The well-known betacyanin is betainin because of its distinctive red color.

Several studies have shown that the free radical scavenging ability of betanin in red beetroot is almost twice as high as that of some anthocyanins under a pH > 4 states (Fu et al., 2020). The ability to fight free radicals and the high antioxidant activity of betanin are related to the presence of
The nutritional content of beetroot includes a high content of vitamin C as an antioxidant. The pink color of beetroot comes from the pigment betacyanin (Nanda, 2014). According to Kusumaningrum, et al (2012), the content of vitamins and minerals in beetroot can stimulate the improvement of the circulatory system and red blood cells. The content of other antioxidants in red beetroot is phenolic with total flavonoids 10.19 ± 1.7 mg/100 g fresh weight and total phenols 323 mg/100 g fresh weight, meaning that the total phenol content is higher compared to other fruits and vegetables. Red beetroot was found to act as a UV protectant (Chen et al., 2021). Antioxidants such as vitamin C and betalain play an important role in protecting the skin against ROS. When the skin is exposed to UV light, ROS compounds such as superoxide and peroxide ions. Antioxidants protect the skin from oxidative stress by successively donating electrons to neutralize free radicals. ROS can trigger chain reactions or cascades that damage cells. The harmful effects of ROS manifest as direct chemical changes in cell DNA, cell membranes, and cell proteins, including collagen. Oxidative stress also triggers certain transcription factor-mediated cellular events, such as B. ROS upregulation of transcription factor activator protein-1 (AP-1), which increases matrix metalloproteinase (MMP) production and leads to collagen degradation. Oxidative stress induces nuclear transcription factor kappa B (NFkB). It produces several mediators that trigger inflammation and skin aging (PK, 2009). ROS also increased elastin mRNA levels in skin fibroblasts. This could explain the changes in elasticity observed in aging skin. Antioxidants are needed to neutralize ROS compounds produced by exposure to UV rays (Lingappan, 2018). Photoaging can be prevented by preventing redness caused by UV radiation, preventing the formation of sunburned cells, and promoting collagen repair. To optimize UV protection, it is important to use sunscreen along with topical antioxidants (Lingappan, 2018). Vitamin C is essential for collagen biosynthesis. Vitamin C affects collagen synthesis quantitatively, in addition to stimulating qualitative changes in the collagen molecule. Vitamin C acts as a cofactor for the enzymes prolyl and lysyl hydroxylase, enzymes responsible for stabilizing and binding the collagen molecule together (Woodby et al., 2017). Previous reports have also shown that the antioxidant activity of betanin is associated with the rich unsaturated bonds in the benzene ring (Esatbeyoglu et al., 2014). As for the specific active functional groups, further research and confirmation are required. Besides containing betanin, red beetroot also contains many polyphenols and phenolics, as small amounts of vitamin C and vitamin E, which have been proven to have strong antioxidant abilities (Kavalcová et al., 2015).

In living cells, free radicals are formed in the plasma membrane, which also occurs in cell organelles such as peroxisomes, endoplasmic reticulum, mitochondria, and cytosol through enzymatic chain reactions arising from metabolic processes. Free radicals are very reactive, can cause chemical changes, and damage various components such as carbohydrates, nucleotides, lipids, and proteins. Under normal conditions, the body can absorb these free radicals because the body naturally produces antioxidants such as catalase and peroxide dismutase. With age, free radicals continue to increase, while natural antioxidants alone are not enough. This creates an imbalance between the free radicals formed and the antioxidants present, leading to the formation of reactive oxygen species (ROS). ROS plays an important role in collagen metabolism. Reactive oxygen species directly destroy interstitial collagen and induce enzymes responsible for collagen degradation, namely matrix metalloproteinases (MMPs), resulting in the loss of dermal collagen (Eickelberg, 2011). Through this pathway, beetroot antioxidants act as external antioxidants that inhibit the formation of MMP-1.

The results of the univariate analysis showed that the group of mice with UVB exposure intervention and given beetroot cream had the highest average amount of collagen, which was 80.08% pixels. compared to the other two observation groups. While the lowest average was in the UVB-exposed group and given basic cream, namely 69.38% pixels. The results of observing the dermis tissue also showed that the P2 observation group (the group exposed to UVB and given beetroot cream) had red collagen fibers that looked wider and thicker which showed collagen expression.

Another study also showed the same results, where UV-B radiation 3 times a week for 6 weeks with a size of 130 MJ/cm² in vivo caused an increase in fibroblast damage in mice (Li et al., 2022). In a study conducted by Wahyono (2020) administration of UVB light at a dose of 130 ml/cm² to 150 ml/cm² reduced the expression of type-1 collagen in the skin of mice aged 4 to 7 months.

Collagen production is influenced by extrinsic factors including ultraviolet light, pollution, and diet (Krutmann et al., 2021). Increased ROS due to free radicals due to UV-B rays can cause increased lipid peroxidation. High levels of free radicals in the body can be indicated by a low activity of antioxidant enzymes and high malondialdehyde (MDA) (Aliaham et al., 2012). This ROS compound also plays a role in collagen metabolism, because besides being able to destroy collagen it can also induce several enzymes that play a role in collagen degradation, namely matrix metalloproteinases (MMPs), resulting in a decrease in skin collagen (Eickelberg, 2011). The accumulation of reactive oxygen compounds will increase MMP-1 and MMP-3. MMP-1 will break down collagen type 1, while MMP-3 can break down collagen type IV, proteoglycans, fibronectin, and laminin. Damage to collagen types I and IV will result in a decrease in collagen production.

Based on the results of the analysis of the mean differences with the one-way ANOVA test, there was a significant difference in the mean amount of collagen in the dermis tissue between untreated rats, treatment group P1 (Group exposed to UVB and given basic ingredient cream), and P2 (Group exposed to UVB and given red beetroot cream). In addition, the correlation test analysis obtained a p-value of 0.000 (p < α) and a correlation coefficient (r) of 0.785, which means that the direction of the correlation between the two variables is positive. This means that the administration of red beetroot extract (Beta vulgaris) inhibits the decrease in collagen with a strong bond strength. This means that the administration of red beetroot cream to rats (Rattus Norvegicus) Wistar strain exposed to UV-B light had a significant effect on inhibiting the decrease in the amount of collagen.
Minimally processed beetroot waste as an alternative source to obtain functional ingredients. Journal of Food Science and Technology, 54, 2050-2059.


2020). Another mechanism by which vitamin C affects collagen synthesis is by stimulating lipid peroxidation, and the product of this process, malondialdehyde, in turn, stimulates collagen gene expression. Vitamin C also directly activates the transcription of collagen synthesis and stabilizes procollagen mRNA, thereby regulating collagen synthesis (Woodby et al., 2020). Clinical studies have shown that topical application of vitamin C increases collagen production in both young and old skin (Boo, 2022). Therefore, based on research results and its content, red beetroot extract cream has the potential to be a topical cream to inhibit the decrease in collagen production in the skin to avoid damage due to exposure to UV-B rays.

V. CONCLUSION

Topical administration of red beetroot (Beta vulgaris) 15% extract cream inhibited the increase in MMP I expression and decreased collagen in the skin of Wistar rats exposed to ultraviolet B light as seen from the mean difference and strong correlation strength on the results of univariate analysis, it can be seen that the groups of mice that were given exposure to UVB and being given beetroot cream had the lowest average MMP1 expression, namely 25.11% and the average amount of collagen was higher, namely as much as 80.08% pixels compared to the other two observation groups.

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CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

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