Terminalia ferdinandiana inhibited the increased of matrix metalloproteinase-I and prevent collagen decreased in mice skin exposed to UV-B

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Abstract

Background: Kakadu cream contained 0,1% Kakadu plum (*Terminalia ferdinandiana*) extract with a high content of phenolic compound, flavonol, tocopherol, lutein, chlorophyll, and ellagic acid has potential to prevent the Ultraviolet-B (UV-B) effect on skin aging acceleration. To date, there's no *in vivo* experiment on the effect of kakadu plum extract on collagen nor matrix metalloproteinase-1 (MMP-1). This research aims to evaluate the effectiveness of Kakadu cream administration in inhibiting the increase of MMP-1 expressions and prevent the decrease of collagen amount in mice (*Mus musculus*) skin exposed to UV-B.

Methods: An experimental study with a post-test only control group design was employed in 36-male-mice, 6-8 weeks old, weighing 20-25 grams. The samples were divided randomly into two groups, a control group given base cream and the study group, given Kakadu cream 0,1% on their shaved backs, 1 cm² in size as the UV-B exposure's location. The UV-B irradiation was done three times a week for 4-weeks. The base and Kakadu cream were given twice a day. Comparative analysis was carried out to compare MMP-1 expression and collagen amount in both groups.

Results: The results show that the mean of MMP-1 expression on the study group was significantly lower compared to control group ($p < 0.001$). The mean collagen amount was significantly higher in the study group than in the control group ($p < 0,001$).

Conclusion: From the results, can be concluded that Kakadu cream inhibited the increase MMP-1 expression and prevent the decrease of collagen amount in mice skin exposed to UV-B.

Keywords: Collagen, MMP-1 expression, Kakadu plum extract, UV-B

Introduction

Ultraviolet (UV) rays exposure is one of the main extrinsic causes of the skin aging process, often called photoaging. Exposure to UV light will cause Reactive Oxygen Species (ROS) formation in the body and damage the dermis, causing a decrease in collagen fibers and fiber solubility and overall thinning the collagen layers.¹ Matrix metalloproteinase-1 (MMP-1) is a type of matrix metalloproteinase that is most responsible for the collagen breakdown in the skin, and its formation

Cite this article:

Emy S, Iswari IS, Winarti NW. *Terminalia ferdinandiana* inhibited the increased of matrix metalloproteinase-I and prevent collagen decreased in mice skin exposed to UV-B. *Neurologico Spinale Medico Chirurgico*. 2021.4(1):11-14. DOI: [10.36444/nsmc.v4i1.136](https://doi.org/10.36444/nsmc.v4i1.136)

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is mostly triggered by exposure to UV light. Aging causes an increase in MMP-1, which leads to fragmentation and disorganization of the collagen fiber arrangement in the skin dermis.² Moreover, UV rays also cause the induction of AP-1 and declines in TGF- β type II and lead to an increase in MMP-1 and a decrease in the amount of collagen.^{2,3}

Many ways are done to reduce skin damage caused by ultraviolet ray exposure such as wearing clothes to avoid direct and excessive sun exposure, using sunscreen creams or lotions that contain antioxidants, or use oral and topical antioxidants.⁴ Naturally, the body will make antioxidants, including superoxidase dismutase (SOD), catalase, and glutathione, as defense factors from free radical exposure. However, natural protection is often less powerful so additional antioxidants from outside the body are needed either orally or topically.

Lately, oral and topical antioxidants usage to prevent and treat aging skin process is increasing and is on trend. Natural antioxidants derived from nature are in great demand because it is believed to provide good benefits with minimal side effects for the skin. The ingredients used to make cosmetic products can come from flowers, leaves, fruit, roots, or other parts of plants.⁵ Kakadu cream contains 0.1% Kakadu fruit extract (*Terminalia ferdinandiana*). Kakadu fruit is a fruit native to Australia, has been widely used by Aboriginal people for hundreds of years because they are known to have many benefits. Previous study shows that Kakadu fruit has the highest vitamin C content, especially compared to other fruits such as blueberries, which have been known to have high levels of vitamin C. Kakadu contains other hydrophilic compounds such as phenolics and flavonoids, and also contains α -tocopherol, γ -tocopherol, δ -tocopherol, lutein, chlorophyll a and b, antioxidants, nutrients, and ellagic acid.⁶

In in vitro research conducted by Lacasse by measuring skin topography using a VISIO-3D Laser Scan (DermaTOP-V3), it was found that Kakadu extract could reduce wrinkles so it can be used as an anti-wrinkle therapy.⁷ Kakadu contains vitamin C with a high concentration, which is useful as an antioxidant that plays a role in neutralizing free radicals due to ultraviolet light exposure and a crucial role in collagen formation. Meanwhile, in vivo studies of Kakadu cream on collagen and MMP-1, the enzymes involved in the degradation of collagen types 1,2, and 3 have not been carried out. For this reason, this research aimed to see the effect of Kakadu cream in inhibiting MMP-1 increased and preventing the collagen decreased thus Kakadu cream may be used as skin aging therapy.

Method

An experimental study using post-test only control group design was conducted in a suitable mice (*Mus musculus*) population. Healthy male mice aged 6-8 weeks and weighed around 20-25 grams are the study inclusion criteria. Following the Federer sampling calculation formula, a total of 36 mice were needed in the study. The samples were randomly divided into two groups—the study and control groups, counting 18 mice in each group. All study procedures were approved by institutional ethic committee with approval number #1506/UN14.2.2VII.14/LT/2020.

The Phillips brand UV-B was irradiated with divided doses: 50 mJ/cm² at the first week, 70 mJ/cm² at the second week, and 80 mJ/cm² at the third and fourth weeks. The irradiation was carried out three times a week for four weeks. Kakadu cream was applied to the back of the mice in the study group evenly with a volume of 0.1 mg/cm² in the irradiated area each time. The cream is taken about 0.1 cc, withdrawn using a syringe then sprayed onto the mice's back then spread evenly using a cotton swab two times a day (20 minutes before and four hours after irradiation). Topical application is still carried out on days without radiation. Meanwhile, the control group was given a base cream material with a similar application procedure to the study group. Mice were left for twenty-four hours after the last exposure to get rid of acute irradiation effects. Kakadu cream is a cream containing 0.1% Kakadu extract, produced by Derma Beauty, Indonesia. The base cream (placebo cream) is a basic cream for the manufacture of creams that do not contain Kakadu fruit extract produced by Derma Beauty Indonesia.

After four weeks of treatment was completed, the skin tissue was taken from the mice's back. The skin was taken using a punch biopsy subcutaneously with a thickness of 3-5 mm and a diameter of 4 mm. The skin obtained was then fixed with a 10% buffer neutral formalin solution and left at room temperature for 48 hours. The MMP-1 enzyme staining used the MMP-1 Kit (*DAKO LSAB plus, universal detection kit, 5ml DAB*) and its expression was calculated using digital analysis with the JPEG format. The tools used are Optilab Viewer 1.0 and Image Raster 2.1 (*miconos Indonesia*). Histological preparations with Sirius red was conducted to assessed the collagen percentage. The collagen calculation was carried out with digital analysis using Adobe Photoshop version-9 paranti.

Data analysis using SPSS 23 (IBM, statistics) was carried out to determine significant differences of MMP-1 and collagen mean in the study and control groups and the correlation between the MMP-1 and collagen. The comparative test analysis was performed using the independent t-test, while the correlation test used Pearson correlation.

Results

The results of the comparison analysis showed that there were significant differences in the MMP-1 and collagen variables in the two groups. The mean MMP-1 in the study group was significantly lower than that in the control group ($p < 0.001$). Meanwhile, the mean of collagen, the study group had a significantly higher than the control group ($p < 0.001$). The results of the comparative test are presented in Table 1.

Table 1. Comparative analysis of MMP-1 and collagen (mean $\pm$ SD) between groups

Variable	Group		p
	Control (n=18)	Study (n=18)	
MMP-1 (%)	29,38 $\pm$ 6,18	10,78 $\pm$ 3,30	< 0,001
Collagen (%)	57,58 $\pm$ 3,72	81,80 $\pm$ 3,30	< 0,001

The Pearson correlation test results showed a strong correlation between the MMP-1 variable and collagen with a significance level of $p < 0.001$. This means that the higher the MMP-1 value, the lower the amount of collagen. The results of the correlation test and correlation coefficient are presented in Table 2.

Table 2. Correlation test of MMP-1 and collagen

Variable	Correlation Coefficient (r)	p
MMP-1- Collagen	-0,971	<0,001

Discussion

This study's final results found that the mean difference was significant in the MMP-1 expression of the study group given 0.1% Kakadu cream, which was lower than the mean of the control group given base cream. Meanwhile, the mean amount of collagen obtained significantly higher in the study group given Kakadu cream than the control group. This shows that giving Kakadu cream for four weeks can inhibit the increase in MMP-1 and prevent the collagen amount decreases in the mice skin exposed to UV-B rays.

This is also in line with research that has been carried out in vitro by Lacasse in 2015 using skin topography Laser Scan VISIO-3D (Derma TOP-V3), and it was found that giving Kakadu extract can reduce wrinkles.⁷ Research conducted using natural ingredients that contain active compounds such as those contained in Kakadu fruit to inhibit the increase of MMP-1 and the collagen decreased conducted by Dianasari (2014) using purple corn extract cream containing active

compounds such as vitamin C, phenolic and anthocyanin in the skin of Wistar rats, it was found that the mean MMP-1 in the control group was 3.22% and the treatment group that was given extract cream was 1.90%, indicating a significant decrease of about 1.32% in MMP-1 levels. On the other hand, on the amount of collagen, the mean control group was 65.54% and the treatment group was 71.70%, which showed a significant increase of around 6.16%. In Kakadu cream, there was a decrease in the mean of MMP-1 by 18.60% and an increase in the amount of collagen by 23.42% which showed a more significant number.⁸

Research conducted by Liliani (2016) using pannax ginseng extract cream containing active compounds in the form of vitamin C, phenolic compounds, and tannins in mice was proven in the results of the study that there was a decrease in the mean value of MMP-1 of around 21.38% between the control and treatment groups and an increase in the mean amount of collagen was around 17.76% between the control and treatment groups. The results of this study show that the mean MMP-1 decreased significantly compared to the results obtained by Kakadu cream, namely with an average MMP-1 of 18.60%, but the amount of collagen in the study on pannax ginseng cream only increased by about 17.76% while the Kakadu cream increased by 23.42%.⁹

Sun exposure will cause collagen changes in the skin dermis in two ways: stimulation of collagen breakdown into fragments and inhibiting procollagen synthesis from reducing collagen content.¹⁰ Irradiation with a dose of 2 MED almost inhibits procollagen formation entirely and lasts for 24 hours, followed by improvement within 48-72 hours after.¹¹ In the previous study, Activator protein-1 (AP-1) and matrix metalloproteinase (MMP) increased and lasted up to 24-hours after exposure to UV light, and collagen breakdown was significantly increased. These MMPs are responsible for degrading protein and are involved in various proteolytic activities in physiological and pathological conditions such as inflammation, wound healing, angiogenesis, embryogenesis, and cancer.¹² MMP-1 is a type of matrix metalloproteinase that is most responsible for the breakdown of collagen in the skin, and its formation is mostly triggered by exposure to ultraviolet-B rays.

Vitamin C functions as an antioxidant that will help neutralize free radicals by donating electrons to unstable compounds to become stable. Oxidative stress does not occur, which will trigger a series of molecular reactions that lead to increased MMP-1 and collagen degradation.¹³ Besides, vitamin C also functions as a cofactor for the enzyme prolyl and lysyl hydroxylase, main enzymes in the process of

forming and stabilize collagen.¹⁴ Phenolic compounds are antioxidants derived from plants. As antioxidants, phenolic compounds work through 3 mechanisms, namely by neutralizing free radicals, suppressing the formation of free radicals by inhibiting enzymes or chelating trace metals involved in the process of free radical formation, and increasing the supply of antioxidants. Phenolic compounds, especially tannins and ellagic acids, have a role in reducing the expression of IL-1 β , IL-6, IL 8, MCP-1 (monocyte chemoattractant protein-1), and TNF- α , which arise due to induction by UVB in vitro. Ellagic acid also functions to inhibit the secretion of metalloproteinases produced by fibroblasts exposed to UVB rays. This can prevent collagen degradation so that the skin's wrinkles become less.¹⁵

Several studies related to the above have also been carried out by Niaimi and Chiang (2017).¹⁴ They researched vitamin C to inhibit the increase in MMP-1 expression where vitamin C inhibits free radicals' formation at the initiation stage, thereby suppressing AP-1 and causing a decrease in MMP-1 expression. Saewan and Jimtaisong (2016) also conducted research showing that the flavonoid compounds in plants can inhibit the formation of free radicals by donating their hydrogen atoms as binders for metal catalysts photoprotection.¹⁶ The theories mentioned above form the basis of research conducted on this Kakadu cream, where the content of vitamin C and phenolic compounds in Kakadu cream can inhibit the increase in MMP-1 and the amount of collagen decreased.

Conclusion

This research evaluated the effect of Kakadu cream extract on the mice skin exposed to UV-B. The administration of 0.1% kakadu cream (*Terminalia ferdinandiana*) can inhibit the increase in MMP-1 expression and prevent the decrease in the amount of collagen in the mice skin (*Mus musculus*) exposed to ultraviolet-B rays. Further research is needed with a longer period of time to assess possible side effects associated with long-term use of kakadu cream. Clinical trials and in vitro studies are needed on human skin to determine the effectiveness of kakadu cream as anti-wrinkle therapy.

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