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The Administration of 1% Broccoli (*Brassica oleracea var. Italica*) Extracts Cream Inhibited the Increase of MMP-1 Expression and Reduction of Dermal Collagen on Male Wistar Rats (*Rattus norvegicus*) Skin Exposed to UV-B Rays

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Abstarct: ***Background*: Aging is a process that every living thing must experience in its life cycle. Various factors are contributed to the aging process, it can be grouped into internal and external factors. One of the external factors is the influence of ultraviolet (UV) radiation that comes from sunlight. Continuous exposure to UV rays can cause photoaging. The effort to prevent photoaging due to exposure to UV radiation is consuming antioxidants which broccoli (Brassica oleracea var. Italica) has excellent antioxidant content. This study was conducted to investigatethat the administration of 1% broccoli (Brassica oleracea var. Italica) extract cream inhibited the increase of MMP-1 expression and reduction of dermal collagen amount in the skin of male Wistar rats (Rattus norvegicus) exposed to UV-B rays. *Methods*: This study was conducted using the post-test only control group design using 36 male Wistar rats as the study sample. The sample was divided into 2 groups, namely the control group and the treatment group. UV-B ray exposure was given three times a week with a total dose of 840 mJ/cm2 over four weeks. The control group was given the basic ingredient cream (placebo cream) and the treatment group was given 1% broccoli extract cream. The cream is given twice a day that is twenty minutes before and four hours after exposure to UV-B rays. Immunohistochemical staining was used to calculate the expression of MMP-1 and to calculate the amount of collagen using staining with Picro Sirius Red then the results were observed and calculated using the software. *Results*:The results of the analysis proceed using the Independent T-Test showed that the mean MMP-1 expression in the control group was significantly higher than the treatment group (26,67 ± 6,724 vs 12,78 ± 3,39) (p< 0,001). Furthermore, the mean amount of dermal collagen in the control group was significantly lower than the treatment group (61,961±4,338 vs 83,4944±2,716) (p< 0,001). *Conclusion*: It can be concluded that administration of broccoli extract cream (Brassica oleracea var. Italica) inhibited the increase of MMP-1 expression and reduction of dermal collagen in male Wistar rats (Rattus norvegicus) exposed to UV-B rays.***

Keywords: broccoli, *Brassica oleracea var. italica*, MMP-1, collagen, UV-B

1. Introduction

Aging is a process that every living thing must experience in its life cycle. The aging process can be prevented, slowed down, and returned to optimal conditions as in youth.[1]

Many factors cause the aging process that can be grouped into internal factors and external factors. One of the external factors that cause aging is environmental influences such as ultraviolet (UV) radiation from sunlight.[1] Direct and continuous exposure to UV rays can induce Reactive Oxygen Species (ROS) and accelerate skin aging or better known as photoaging.[2,3] This process will lead to the expression of a matrix metalloproteinase (MMP).

Matrix metalloproteinases such as collagenase cause collagen degradation and inhibit new collagen synthesis.[4,14] Efforts to prevent photoaging are consuming antioxidants.[4.5]

Antioxidants are substances that slow down, prevent, or eliminate molecular damage due to oxidation reactions that cause the formation of free radicals.[6] Broccoli is a source of antioxidants that contain lots of flavonoids, phenols, and tannins, which can prevent skin damage due to exposure to UV-B rays.If the skin aging process could be prevented, the quality of life will improve. Therefore, we are interested in studying the effect of 1% broccoli extract cream on MMP-1 expression and the amount of collagen.

2. Study Method

Design and sample study

This study was conducted with the post-test only control group design with 36 male Wistar rats as study subjects. The study subjects were healthy rats aged 10-12 weeks weighing 180-200 grams. Subjects were randomly divided into two, namely the control group and the treatment group (n = 18).

Subject intervention

The rats' hair was shaved around the area to be applied the cream to optimize penetration and the cream was applied twice a day, twenty minutes before exposure, and four hours after irradiation. Ultraviolet B exposure at a total dose of 840 mJ/cm^2 was given three times a week for four weeks. The control group received the basic ingredient cream without extract (placebo) and the treatment group received 1% broccoli extract cream. The cream remains applied on days without exposure to UV-B rays. After four weeks, all mice were anesthetized and skin samples were taken from the back through a biopsy.

MMP-1 expression calculation

MMP-1 expression was stained using immunohistochemical techniques. The stained tissue was divided into three fields of view and observed using a 400x magnification light microscope. Expression of matrix metalloproteinase-1 was calculated as the mean of fibroblasts expressing MMP-1 (brown cytoplasm). The results were then analyzed using an independent t-test.

Dermal collagen calculation

To determine the amount of collagen, tissue stained with Picro Sirius Red was then observed using a 40x magnification light microscope. The sample images were analyzed using Adobe Photoshop CS3 and Image J. The percentage of dermal collagen is obtained by dividing the area of collagen pixels by the total pixels in one field ofview. The results were then analyzed using an independent sample t-test.

3. Results

Table 1: Comparisons between Groups

Variable	Groups	N	Mean ±SD	IntervalMean (CI 95%)	p
MMP-1 expression (%)	Control	18	26,67±6,724	13,89 4(10,239 s/d 17,549)	<0,001
	Treatment	18	12,78±3,39		
Collagen Amount (%)	Control	18	61,961±4,338	-21,533(-24,002s/d -19,064)	<0,001
	Treatment	18	83,494±2,716		

n= Number of samples, SD= Standard Deviation

Figure 1 shows the difference between the control and treatment groups. MMP-1 expression was lower in the treatment group, while the amount of dermal collagen was higher in the treatment group. Data between the treatment and control groups were analyzed using an independent sample t-test and the mean differences between groups are presented in Table 1. The mean MMP-1 expression in the control group was higher than the treatment group (26,67 ± 6,724 vs 12,78 ± 3,39, p< 0,001). Furthermore, the mean amount of dermal collagen in the control group was lower than the treatment group (61,961±4,338 vs 83,494±2,716)(p <0,001).

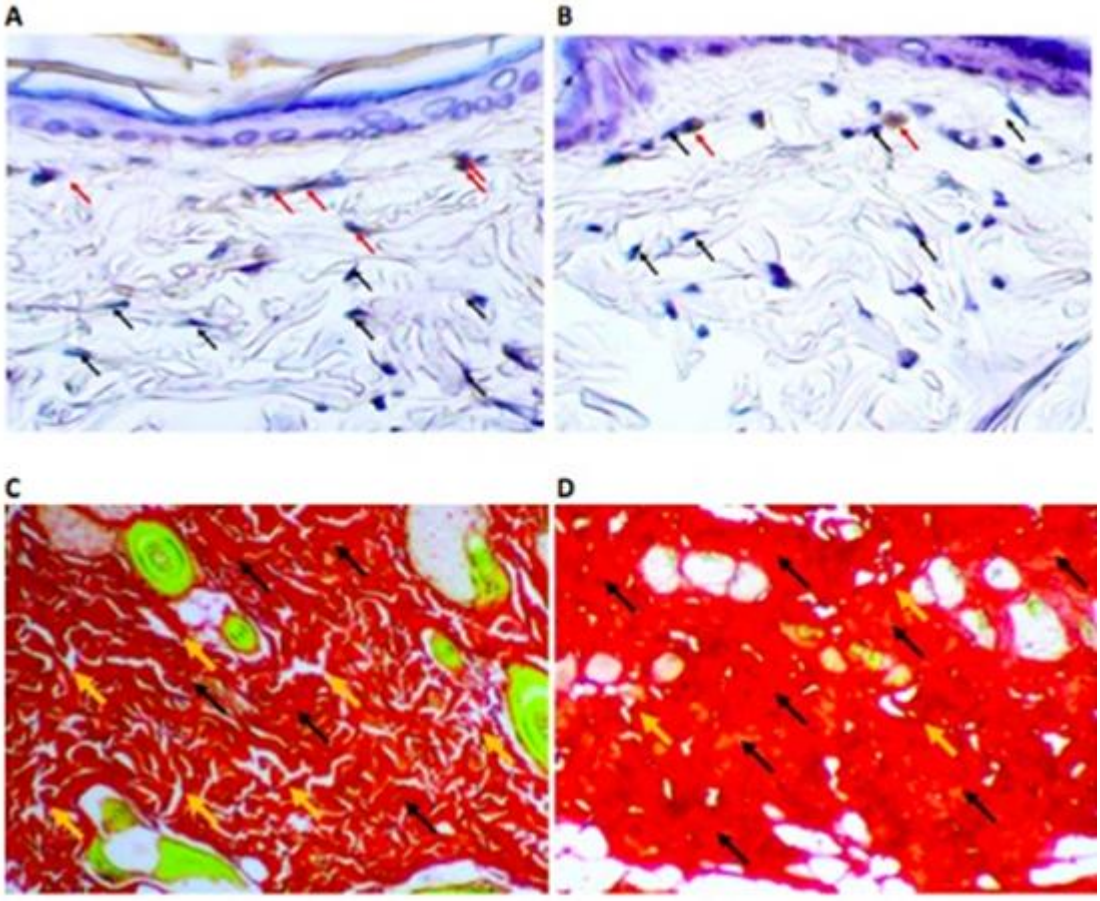

Figure 1: Figure **A**. Control Group (400x Magnification). It appears that the expression of MMP-1 (brown color) is increased compared to other groups. Red arrows indicate fibroblast cells expressing MMP-1. Black arrows indicate fibroblast cells that do not express MMP-1. Figure **B.** The treatment group given 1% broccoli extract cream (400x magnification) shows that MMP-1 expression (brown color) is less than Figure A. Red arrows indicate fibroblast cells expressing MMP-1. Black arrows indicate fibroblast cells that do not express MMP-1. Figure **C.** Control Group (40x magnification). There is damage to the composition and structure of collagen with red collagen fibers appeared thin. The black arrows indicate thick collagen fibers.

Yellow arrows indicate collagen fibers that are not thick. Figure **D.** Treatment group given 1% broccoli extract cream (40x magnification) There was an increase in the composition and structure of collagen with thick red collagen fibers. The black arrows indicate thick collagen fibers. Yellow arrows indicate collagen fibers that are not thick

4. Discussion

This study shows that 1% broccoli extract cream can prevent photoaging of the skin, as evidenced by the lower expression of MMP-1 and a higher amount of dermal collagen in the treatment group. Phytochemical results show that broccoli contains high levels of phenols, flavonoids, and tannins which are useful as antioxidants to inhibit skin damage due to UV rays.

UV exposure activates Reactive Oxygen Species (ROS), which causes skin aging.[4] The process begins with the activation of keratinocyte and fibroblast cell surface receptors and surface cytokines stimulates the signal transduction. Later c-Fos and c-Jun transcription factors through heterodimerization and AP formation -1 become real. The MAPK transduction pathway also stimulates the transcription factor AP-1 and the transcription process of several MMPs such as MMP-1, MMP-2, MMP-3 which degrade the extracellular matrix. Furthermore, transcription factor AP-1 inhibits collagen type 1 transcription genes, damages the extracellular matrix and tissue integrity, and reduces the expression of extracellular matrix structural proteins.At the same time, there is a decrease in collagen synthesis due to inhibition of the Transforming Growth Factor Beta (TGF-P) receptor which increases collagen production.[4,8]

Flavonoids contained in broccoli can suppress matrix metalloproteinase-1 (MMP-1) expression and induce procollagen type I protein expression. Also, flavonoids prevent phosphorylation of the cascade components of mitogen-activated protein kinases (MAPK), namely ERK, JNK, and c-Jun involved in the induction of MMP-1 expression after exposure to UVB. Furthermore, flavonoids influence the TGF-β / Smad signaling pathway, which is involved in the regulation of type I procollagen expression.[7]Phenolic compounds work as chain-breaking antioxidants or scavenger antioxidants, by releasing one hydrogen atom from the hydroxyl group so that ROS is not formed.[9]Tannins are polymer compounds of high molecular weightpolyphenols. The working mechanism of tannins in inhibiting dermal collagen damage due to UV exposure is to inhibit MMP-1 activation through inhibition of AP-1.[10,11]

In this study, it was shown that the expression of MMP-1 in the control group was significantly higher than the treatment group (26,67 vs 12,78). This shows the role of broccoli in reducing the adverse effects of exposure to UV rays as an antioxidant. The treatment group has significantly higher dermal collagen than the control group (83,50% vs 62,00%).The benefits of topical plant extracts to inhibited increased expression of MMP-1 and decrease the amount of dermal collagen have been previously demonstrated by Wiraguna et al, where administration of purple corn extract cream can also inhibit the increase of MMP-1 after UV-B exposure. MMP-1 expression in the control group was higher than the treatment group (3.22 vs 1.90). Meanwhile, the amount of dermal collagen in the treatment group had higher dermis collagen than the control group (71.70% vs 65.541%).[12] Giving extract of Caulerpa sp. can also prevent increased expression of MMP-1 and decreased amount of dermal collagen. MMP-1 expression in the control group was higher than the treatment group (13.22 vs 5.52). While the amount of dermal collagen in the treatment group had higher dermal collagen than the control group (58.91% vs 43.45%). [13]

5. Conclusion

This study proves the anti-aging potential of 1% broccoli extract cream by inhibiting MMP-1 expression and decreasing the amount of skin collagen caused by UV-B irradiation in male Wistar rats.

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