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ADMINISTRATION OF *BAJAKAH* (*SPATHOLOBUS LITTORALIS HASSK*) STEM ETHANOL EXTRACT CREAM INHIBITED THE INCREASING OF MMP-1 EXPRESSION AND THE REDUCING OF COLLAGEN IN MALE WISTAR RATS (*RATTUS NORVEGICUS*) EXPOSED TO ULTRAVIOLET B

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ABSTRACT

Background: *Exposure to ultraviolet B (UV-B) rays causes skin aging characterized through increased free radical damaged. To prevent skin aging due to UV-B exposure, it is necessary to additionally provide antioxidants as a measure to inhibit the skin aging process. Bajakah tree (Spatholobus littoralis hassk) is one antioxidants sourche such as phenols, flavonoids, and tannins. The aim was to prove that the administration of Bajakah stem ethanol extract cream inhibits increasing MMP-1 expression and reduction of collagen number in male Wistar rats exposed to UV-B.*

Methods: *A posttest control group study design was coducted in male rats (Rattus norvegicus), Wistar strain, aged 2-3 months, weigh 180-200gram which was divided randomly into two groups. The control group (n = 18) was exposed to UV-B rays and placebo cream (0.2 mg/cm^2), while the treatment group (n = 18) was exposed to UV-B rays and Bajakah stem ethanol extract cream 15 % (0.2 mg/cm^2). Both creams have aplied twice before and after exposure to UV-B rays (3 times a week, with 840 mJ/cm^2) for 4 weeks. Twenty-four hours after the last day intervention, a punch biopsy of skin tissue was prepared for histological examination followed by immunohistochemical assay (for MMP-1) and Sirius red (for collagen) staining.*

Results: *The expression of MMP-1 was significantly higher in the control group (24.3 ± 6.20%) compared to the treatment group (11.5 ± 3.21%), p<0.001. Furthermore, the number of collagen was statistically lower in the control group (63.1 ± 3.94%) compared to the treatment group (82.0 ± 3.02%), p<0.001.*

Conclusion: *The administration of bajakah (Spatholobus littoralis hassk) stem ethanol extract cream inhibited the increasing of MMP-1 expression and the reducing of collagen cells in male Wistar rats (Rattus norvegicus) those were exposed to UV- B.*

Keywords: *Bajakah stem, MMP-1, collagen, ultraviolet B.*

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INTRODUCTION

Indonesia is one of tropical countries with high sunlight intensity that can cause skin aging. Sunlight caused both acute and chronic effects on the skin. In this current study, what was observed was chronic damage, namely photoaging. Inhibiting, preventing, and restoring the skin aging process can be done and is one of the focuses of Anti-Aging Medicine (AAM). Since ultra violet (UV) rays in sunlight exposure causes skin aging, efforts to prevent the development of the aging phenotype is one of the Anti-Aging Medicine.[1]

Sunlight that caused photoaging is UV rays with a wavelength of 10 - 400 nm. Photoaging is characterized by an increase expression of matrix metalloproteinase (MMP).[2] MMP-1 is the main collagenase increased by UV-B exposure. UV radiation induces MMP-1 expression by

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dermal fibroblasts, partly stimulated by the formation of excess reactive oxygen species (ROS), and plays an important role in photoaging.[3] MMP-1 together with its inhibitor, tissue inhibitor of metalloproteinases (TIMPs), plays a role in the fragmentation of types I and III collagen. The increasing MMP-1 due to UV-B resulted in the increasing collagen degradation, so that the amount of collagen decreases. The molecular mechanism of MMP-1 degrades collagen is by cutting the collagen fibers in the three alpha chains at one locus which is located around the first quarter of the N-terminal collagen chain and produces TCA and TCB fragments.[4]

An increase of MMP-1 and be followed by a decrease of collagen are the main causes of skin aging due to exposure of UV-B rays; hence, it is necessary to make efforts to inhibit the increase in MMP-1 and the decrease of collagen as part of AAM. Many studies have been carried out using natural creams containing antioxidants and bioactive compounds to prevent photoaging.

Recently, the stem of *Bajakah* tree (*Spatholobus littoralis hassk*), a plant that is growing in forest of Kalimantan island, has come to attention in Indonesia.[5] Traditionally, this plant has been used orally or topically by the local community for curing some health problems.[6] Previously, research has shown qualitatively the presence of flavonoids, saponins, steroids, terpenoids, tannins, and phenols, and has been shown to accelerate the wound healing process.[5]

Scientific research on the Bajakah plant is only limited to its qualitative content and its effect on the wound healing process.[5] The content of active compounds and antioxidants have been widely reported to be able to prevent photoaging. The purpose of the study was to prove that the administration of Bajakah stem ethanol extract cream inhibits the increasing of MMP-1 expression and the reducing of collagen cells in male Wistar rats exposed to UV-B.

METHODS

A post-test only control group design, with subjects male rats (*Rattus norvegicus*), Wistar strain, aged 2-3 months, weighing 180-200gram which were divided randomly into two groups. The control group (n = 18 rats) was exposed to UV-B rays and placebo cream (0.2 mg/cm^2), while the treatment group (n = 18 rats) was exposed to UV-B rays and Bajakah stem ethanol extract cream 15 % (0.2 mg/cm^2). The cream was administered 20 minutes before and 4 hours after exposure to UV-B rays (3 times a week, a total dose of 840 mJ/cm^2), for four weeks. Twenty-four hours after the last day of intervention, a punch biopsy of skin tissue was prepared for histological examination followed by immunohistochemical assay (for MMP-1) and Sirius red (for collagen) staining.

RESULTS

The expression of MMP-1 in the control group was (24.3 ± 6.20)% and the treatment group was (11.5 ± 3.21)%. The comparative analysis using the independent *T*-test showed a *p*-value of <0.001 which indicates that there was a significant difference in MMP-1 expression higher in the control group compared to the treatment groups (pigure 3.(A)). In addition, the amount of collagen in the control group was (63.1 ± 3.94)% and the treatment group was (82.0 ± 3.02)%. Comparative analysis showed a *p*-value of <0.001 which indicates that there was a significant difference in the amount of collagen lower in the control group compared to the treatment groups.

The Visual fiture shown in pigure 1, the experssion MMP-1 (brown color) in the control group (A, B) is higher than that in the treatment group (C, D). So do, the collagen fiture in pigure 2 shown that collagen of the control group (A, B) fragmented and tinner than that the collagen of the treatment group (C,D).

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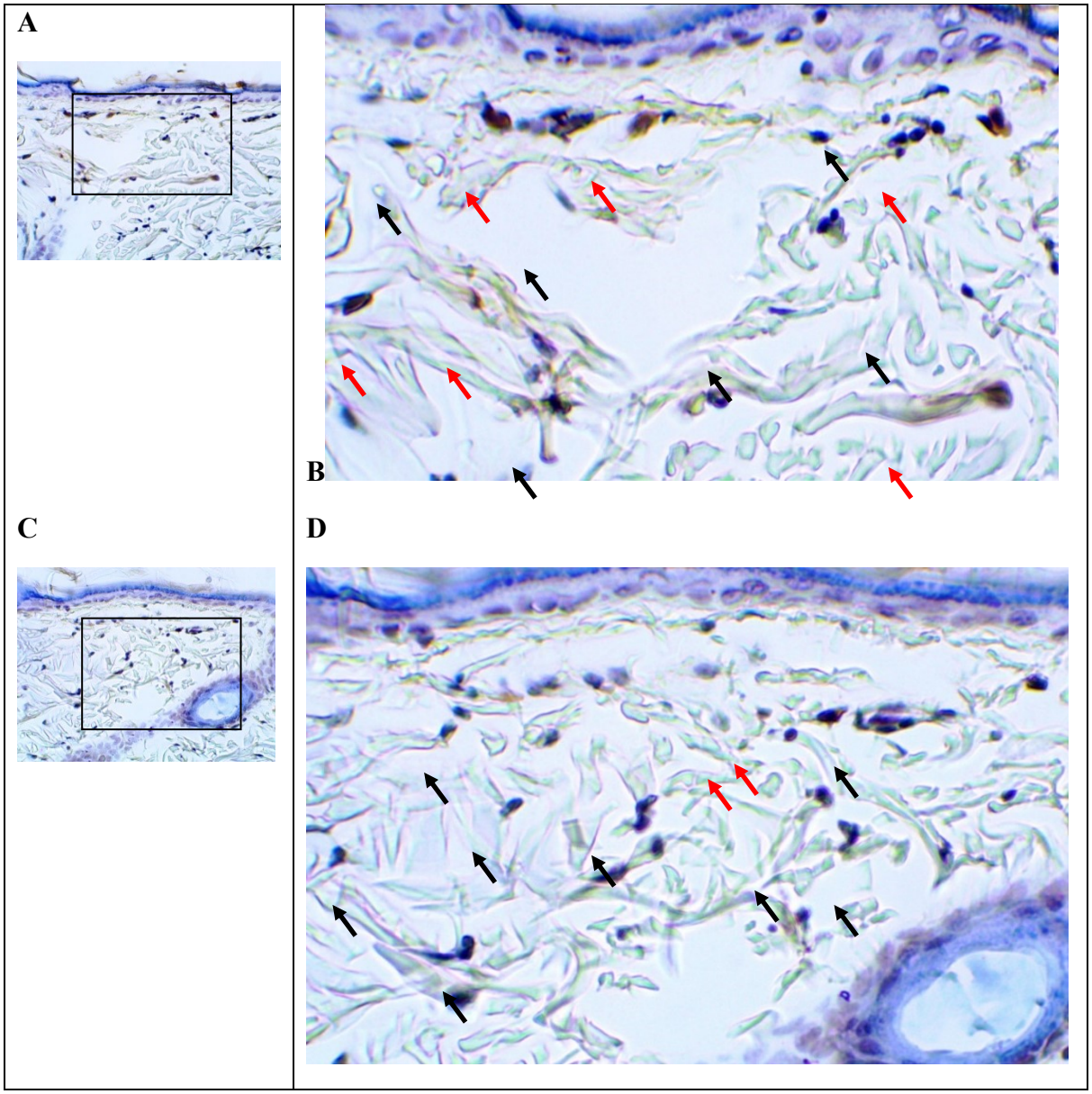

Figure 1. Expression of MMP1 examined by immunohistochemistry in Dermal Tissue

A. Control group (100x magnification).
B. Control group (400x magnification). The expression of MMP-1 (brown color) was higher compared to the treatment groups. Red arrows indicate fibroblast cells expressing MMP-1. Black arrows indicate fibroblast cells that did not express MMP-1
C. Treatment group (100x Magnification)
D. Treatment group (400x magnification). MMP-1 expression (brown color) was less than the control group. Red arrows indicate fibroblast cells expressing MMP-1. Black arrows indicate fibroblast cells that did not express MMP-1.

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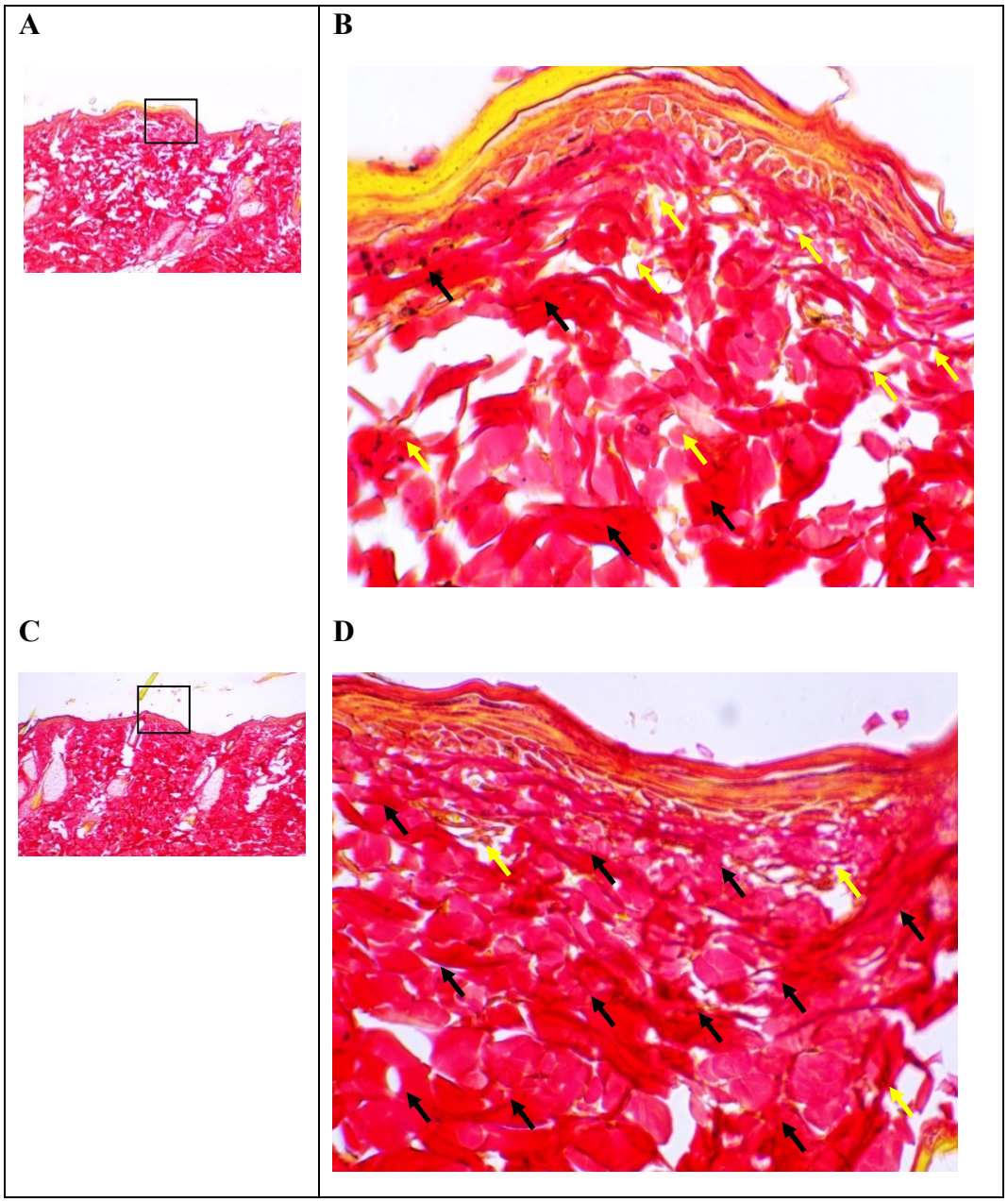

Figure 2. Collagen in Dermal Tissue with Picro-Sirius Red Staining

A. Control group (100x magnification).
B. Control group (400x magnification). The structure of collagen with red collagen fibers appeared to disintegrate and thin. The black arrows indicate thick collagen fibers. Yellow arrows indicate thin collagen fibers.
C. Treatment group (100x Magnification).
D. Treatment group (400x enlargement). The collagen structure was more intact and thick than that of the control group. The black arrows indicate thick collagen fibers. Yellow arrows indicate thin collagen fibers.

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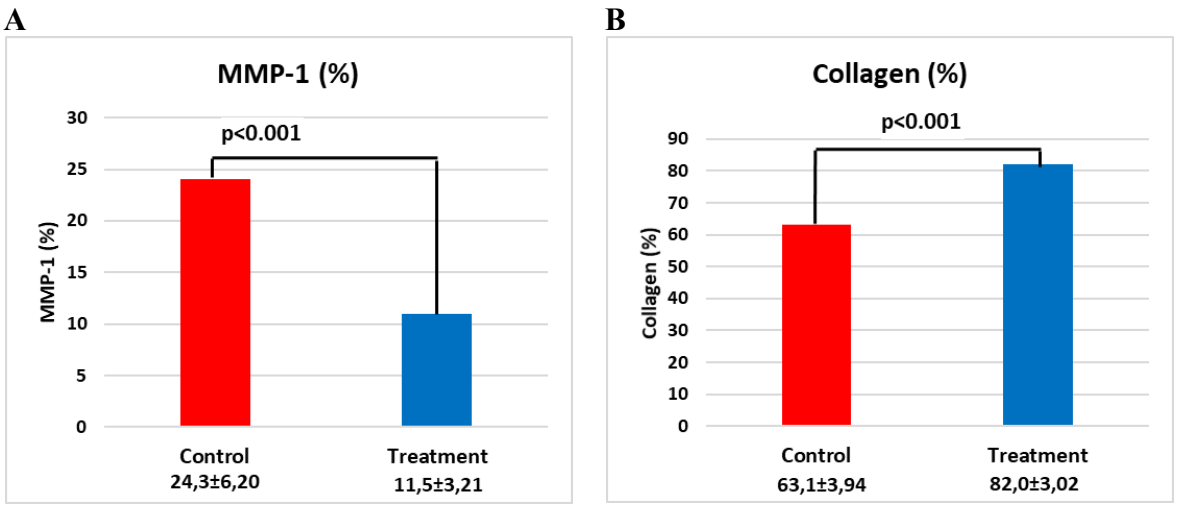

Figure 3. The Comparison of (A) MMP-1 Expressions (%) and (B) Collagen number (%) between Control and Treatment Groups

DISCUSSION

Recently, the Bajakah plant (*Spatholobus littoralis hassk*) receives a lot of attention in Indonesia because of its potential to inhibit the growth of cancer cells.[5] In addition, this plant has been widely used traditionally, for some health problem.[6] However, the current study is the first to demonstrate the potential of the ethanol extract of the stem of bajakah for preventing aging process, particularly for skin aging caused by exposure to UV-B rays.

Anti-Aging Medicine (AAM) aims to maintain health regardless of chronological age i.e. to stay healthy and biologically efficient. AAM aims to treat the causes of aging that underlie the aging process and to reduce all age-related diseases.[1] One aspect of aging that many people focus on is skin aging. By knowing the etiology and pathophysiology of skin aging, efforts can be made to prevent this skin aging process.

Previously, research has shown qualitatively the presence of flavonoids, saponins, steroids, terpenoids, tannins, and phenols, and has been shown to accelerate the wound healing process in mice.[5] These results were then confirmed through the results in this study, showing that the phytochemical content of the Bajakah stem extract were flavonoids, phenols, tannins, and antioxidant capacity. Each of these active compounds contained in the Bajakah stem ethanol extract has their respective contributions and roles in inhibiting the increase in MMP-1 expression and decreasing the amount of collagen.

Polyphenols have a photoprotective effect on oral and topical administration through their antioxidant abilities.[8] As antioxidants, phenolic compounds remain stable and do not experience resonance after donating atoms in radical compounds, thus stopping chain reactions caused by other radicals.[9] Because UV-B radiation increases the production of ROS which then activates MAPK and forms complex with the transcription factor AP-1, which plays an important role in the regulation of MMP-1 transcription which then results in collagen degradation.[10] Hence, polyphenols in the ethanol extract of the Bajakah stem which are antioxidants can neutralize the production of ROS due to UV-B and there is no increase in MMP-1 expression, and collagen degradation is also inhibited.

Research has reported that flavonoids can suppress MMP-1 expression and induce expression of procollagen type I protein in UV-induced cell culture.[11] Flavonoids also inhibit

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the activation of nuclear factor kappa B (NFkB), which is a transcription factor for MMP-1 so that MMP-1 levels decrease and collagen degradation does not occur.[12]

Research has shown that tannins can interact with collagen through hydrogen bonds and hydrophobic interactions thereby increasing the thermal stability and enzymatic stability of collagen. Tannins can increase the hydrothermal stability of collagen and inhibit collagen degradation by MMP-1 through the formation of hydrogen bonds and hydrophobic interactions. Tannins also bind to collagen with high affinity because the structural flexibility of collagen compensates for the structural rigidity of phenolics.[13]

To date, many studies have been carried out using natural ingredients creams that contain antioxidants and bioactive compounds to prevent photoaging. This is because Indonesia has vast natural resources that are easily available and relatively cheap. In addition, natural compounds are relatively safer to use (relatively lower toxicity) than synthetic chemicals.

This research was conducted because we believed that the Bajakah stem extract had better potency than other plant extracts that had been studied previously. Bajakah stem extract cream contains phytochemical compounds and antioxidants that are better than other plants. The extract of the Bajakah stem used in this study had flavonoid content (79739.70 mg/100gQE), total phenol (14952.12 mg/100gGAE), tannins (17920.42 mg/100gTAE), antioxidant capacity (63141.06 mg/L) and Inhibitory Concentration (IC) 50% (13.25 mg/L).

Whereas previous research using cherry leaf extract cream (*Muntingia calabura* Linn) contained a total phenol of 2352.77 mg/100gGAE, flavonoids of 1765.34 mg/100gQE, tannins of 289.50 mg/100gTAE, antioxidants of 7563.90 mg/L GAEAC, and IC50% of 53.18 ppm were sufficient to inhibit the increase in MMP-1 expression and decrease the amount of collagen in the skin of male Wistar rats exposed to ultraviolet B rays.[14] The *Lepisanthes amoena* leaf extract containing flavonoids (986.62 mg/100gQE), antioxidant capacity (135627.21 mg/L), and IC50% (101.25 mg / L) can inhibit the increase in MMP-1 and decrease the amount of collagen in male Wistar rats exposed to UV-B rays.[15]

Based on the comparison of these bioactive compounds, it can be concluded that the Bajakah stem extract cream is potentially better for use as an Anti-Aging Medicine, especially skin aging caused by exposure to UV-B rays.

Excessive UV-B exposure is the main etiology of skin aging especially in tropical countries like Indonesia. Meanwhile, an increase in MMP-1 and a decrease in the amount of collagen is pathophysiology of skin aging due to exposure to UV-B rays. So that in relation to AAM it is necessary to make efforts to inhibit the increase in MMP-1 and decrease the amount of collagen. Bajakah stem ethanol extract cream is one of the Anti-Aging Medicine steps because it can prevent and inhibited the pathophysiology of skin aging.

CONCLUSION

The administration of Bajakah (*Spatholobus littoralis hassk*) stem ethanol extract cream inhibited increasing MMP-1 expression and the reduction of collagen in male Wistar rats (*Rattus norvegicus*) exposed to ultraviolet B. Next, it is necessary to perform a comparative study of the Bajakah stem cream with creams of other plant extracts with the same content to prove that natural ingredients are the best anti-aging modality for the skin. However, the toxic potential for long-term topical use ethanol extract of the Bajakah stem has never been reported; thus, further study is necessary. Clinical research in humans is also warranted before it can be used widely in society.

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