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Administration of Bajakah (*Spatholobus littoralis Hassk*) Stem Ethanol Extract Cream Inhibited the Increase of MMP-1 Expression and Decrease of Collagen Number 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 by an increase in the expression of matrix metalloproteinase-1 (MMP-1) and a decrease in the amount of collagen through increased free radical production. 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 in Wistar rats exposed to ultraviolet B rays. One source of antioxidants is a natural ingredient such as bajakah (Spatholobus littoralis hassk) stem. The ethanol extract of the Bajakah stem contains antioxidants such as phenols, flavonoids, and tannins. The purpose of this study was to prove that the administration of Bajakah stem ethanol extract cream inhibits the increase MMP-1 expression and decrease of collagen number in male Wistar rats exposed to UV-B. Methods: This study used a post-test only control group design. Subjects were 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, the total dose of 840 mJ/cm^2). Twenty-four hours after the last radiation, 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 between the control and treatment groups. 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 between the control and treatment groups. Conclusion: Based on the results of this study, it can be concluded that administration of bajakah (Spatholobus littoralis hassk) stem ethanol extract cream inhibited the increase of MMP-1 expression and decrease of collagen number in male Wistar rats (Rattus norvegicus) exposed to ultraviolet B.***

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

1. Introduction

Indonesia is a tropical country with high sunlight intensity that can cause skin aging. Sunlight can cause acute (erythema, connective tissue damage, DNA mutation, inflammation) and chronic (premature skin aging or photoaging and skin cancer or photocarcinogenesis) effects on the skin. In this 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 UV exposure causes skin aging, efforts to prevent the development of the aging phenotype due to UV rays is one of the Anti-Aging Medicine[1]

Sunlight that can cause photoaging is ultraviolet (UV) with a wavelength of 10 - 400 nm. Photoaging is characterized by an increase in matrix metalloproteinase (MMP) expression.[2] MMP-1 is the main collagenase increased by UV-B exposure. UV radiation induces MMP-1 expression by 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 results in increased 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 in MMP-1 and a decrease in the amount of collagen are the main causes of skin aging due to exposure to UV-B rays; hence, it is necessary to make efforts to inhibit the increase in MMP-1 and decrease the amount 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 Bajakah (*Spatholobus littoralis hassk*) stem has come to attention in Indonesia. [5]Traditionally, this plant has been used orally or topically. [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 this study was to prove that the administration of Bajakah stem ethanol extract cream inhibited the increase of MMP- 1 expression and decrease of collagen number in male Wistar rats exposed to UV-B.

2. Methods

This study used a post-test only control group design. Subjects were 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). Twenty-four hours after the last radiation, a punch biopsy of skin tissue was prepared for histological examination followed by immunohistochemical assay (for MMP-1) and Sirius red (for collagen) staining.

3. 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 between the control and treatment groups. 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 between the control and treatment groups.

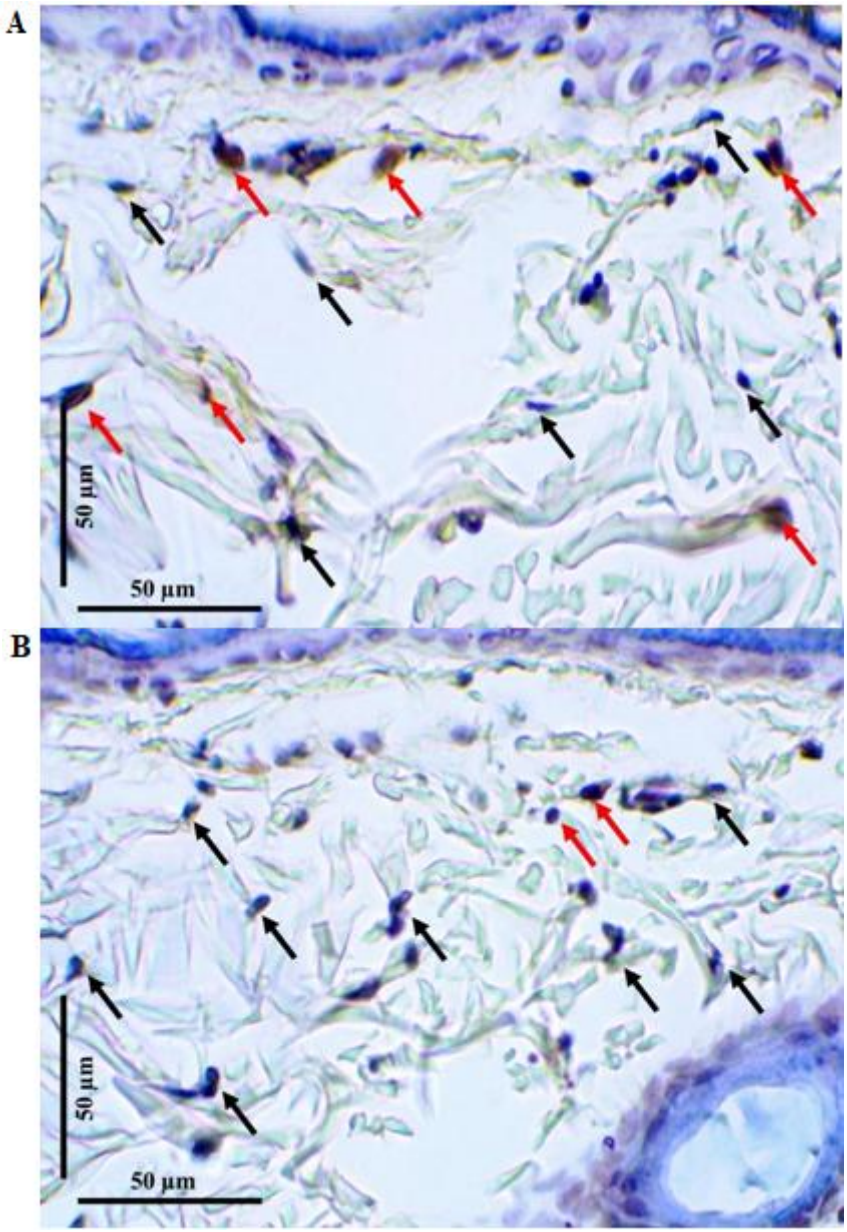


Figure 1: Expression of MMP1 examined by immunohistochemistry in Dermal Tissue

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.

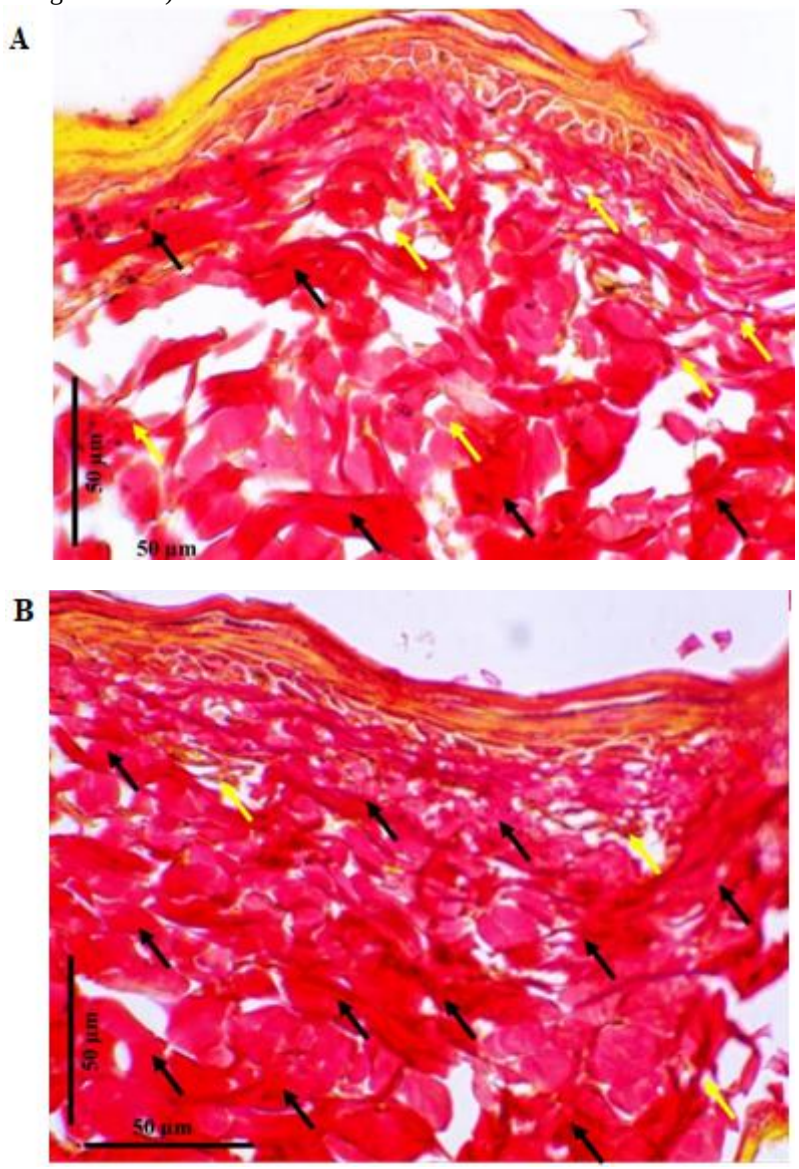
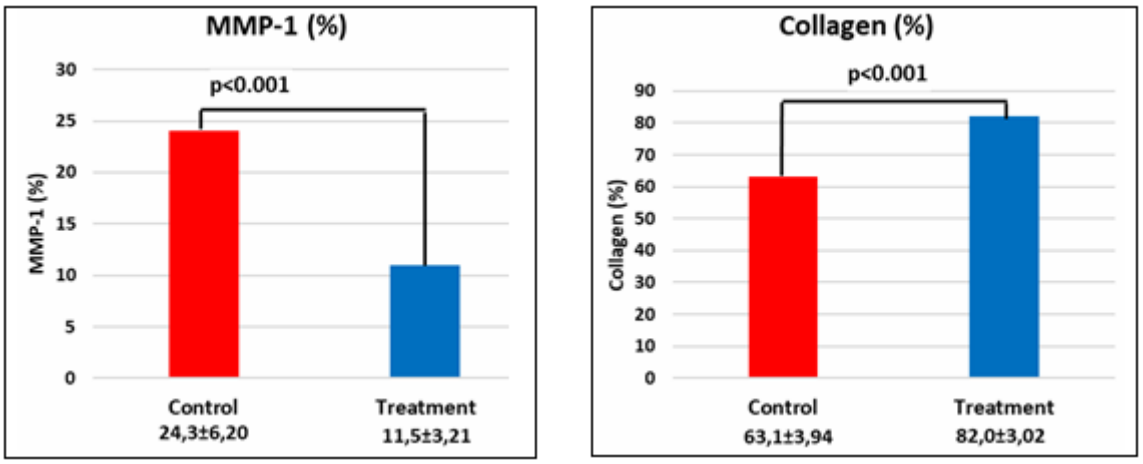


Figure 2: Collagen in Dermal Tissue with Picro-Sirius Red Staining

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.



(A) **(B)**

Figure 3: Graphical Comparison of (A) MMP-1 Expressions and (B) Collagen Amount between Control and Treatment Groups

4. Discussion

Research subjects

To prove that administration of Bajakah stem ethanol extract cream inhibited the increase of MMP-1 expression and decrease of collagen number in male Wistar rats exposed to UV-B, experimental research was carried out using the post-test only control group design. The subjects used as experimental animals in this study were male rats (*Rattus norvegicus*), Wistar strain, 2-3 months old, weighing 180-200 grams.

The experimental animal used was rats because it has several advantages, including inexpensive, easy to obtain, requiring little space, and easy maintenance. [6]The rats used was *Rattus norvegicus* because its body is bigger than *Rattus rattus*[7], so it has a larger and better body surface area for this study which used a skin biopsy as a sample. The *R. norvegicus* strains which have been widely used in research are the Wistar and Sprague Dawley. Biologically, these two strains are not different, so the selection of the Wistar strain in this study does not have a specific reason related to the observed variables. [7]The use of male sex was because male rats are not affected by the menstrual cycle as in female Wistar rats, where changes in 17β-Estradiol and Progesterone hormones occurred which will affect the amount of MMP-1 and collagen so that it can affect the results of the study.

Effect of Bajakah Stems Extract Cream on MMP-1 and Collagen

The results of this study indicated that the expression of MMP-1 in the treatment group was significantly lower than in the control group. The amount of collagen between groups was also significantly different ($p < 0.001$), with the amount of collagen in the treatment group being significantly higher than the control group. 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.[6] However, this study is the first to demonstrate the potential of the ethanol extract of the stem of bajakah as an Anti-Aging Medicine, particularly for skin aging caused by exposure to UV-B rays.

Mechanism of Bajakah Stems Extract Cream toward MMP-1 and Collagen

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 showed that flavonoids can suppress MMP-1 expression and induce expression of procollagen type I protein in UV-induced cell culture. [11]Flavonoids also inhibit 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 can bind to collagen with high affinity because the structural flexibility of collagen compensates for the structural rigidity of phenolics.[13]

Comparison of Bajakah Stem Extract with Other Extracts

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 the researchers 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 (986.62mg/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.

Bajakah Stem Extract as Anti-Aging Medicine

Anti-Aging Medicine (AAM) aims to maintain health regardless of chronological age 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.

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. In this study, it was concluded that administration of Bajakah (*Spatholobus littoralis hassk*) stem ethanol extract cream inhibited the increase of MMP-1 expression and decrease of collagen number in male Wistar rats (*Rattus norvegicus*) exposed to ultraviolet B; hence, Bajakah stem ethanol extract cream is one of the Anti-Aging Medicine steps because it can prevent the pathophysiology of skin aging.

5. Conclusion

Based on the results of this study, it can be concluded that administration of Bajakah (*Spatholobus littoralis hassk*) stem ethanol extract cream inhibited the increase of MMP-1 expression and decrease of collagen number 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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