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Original Article

In vivo study of *Centella asiatica* leaf extract cream for Matrix Metalloproteinase-1 inhibition and collagen enhancement to prevent photoaging

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A B S T R A C T

Background: Ultraviolet B (UVB) exposure accelerates skin aging, causing wrinkles and increasing matrix metalloproteinase-1 (MMP-1) activity, which degrades collagen. *Centella asiatica* (CA) has well-documented anti-inflammatory and antioxidant properties that may counteract UVB-induced photoaging. However, studies on its topical formulation for MMP-1 inhibition and collagen enhancement remain limited.

Purpose: This study aims to determine the effect of CA leaf extract cream on inhibiting MMP-1 and increasing collagen in skin tissue exposed to UVB rays.

Methods: This experimental study used a post-test only control group design. The sample population of BALB/c mice was 24, divided into four groups: healthy control group (KN), negative control group (K-), Treatment 1 (P1): 10% CA extract cream dosage, and Treatment 2 (P2): 20% CA extract cream dosage. Skin tissue samples were measured for MMP-1 expression using immunohistochemistry (IHC) staining, and collagen density using Sirius Red staining. Data analysis on MMP-1 expression using One-way ANOVA test and collagen density using Kruskal Wallis test.

Results: MMP-1 expression was highest in the K2 group (UVB exposure + cream base) and decreased in the P1 and P2 groups (10% CA cream), with the lowest expression in the K1 group (healthy control). However, One-Way ANOVA showed no significant differences among groups (p=0.053). Collagen density analysis using the Kruskal-Wallis test showed significant differences (p = 0.04), with a decrease in the K2 group and an increase in the K3 and K4 groups.

Conclusion: Application of CA extract cream affects MMP-1 expression and collagen density in skin tissue exposed to UVB rays.

INTRODUCTION

Photoaging refers to premature skin aging caused by prolonged exposure to ultraviolet (UV) radiation, particularly UVB, from the sun. Skin thinning, sagging, fine wrinkles, and actinic elastosis characterize it. The incidence of photoaging has been increasing over the years.[1] In Indonesia, 57.3% of cases are attributed to UV exposure, with xerosis (dry skin) affecting 50%–80% of the population. Globally, the prevalence of xerosis due to photoaging ranges from 35% to 80%.[2] Studies indicate that approximately 72% of men and 42% of women under 30 experience photoaging.[3]

Several studies have highlighted the potential of *Centella asiatica* (CA) leaf extract as an antioxidant, anti-inflammatory, and regenerative agent that can repair skin damage.[1,4] UVB exposure induces various adverse effects on the skin, ranging from sunburn to wrinkle formation, by increasing reactive oxygen species (ROS) levels, triggering inflammation, and causing DNA damage. These molecular changes lead to elevated matrix metalloproteinase-1 (MMP-1) activity, accelerating collagen degradation.[5,6] Anti-aging skin care products are commonly formulated with antioxidants to protect against free radical-induced damage.[7,8] Natural ingredients are increasingly favored in topical applications due to their minimal side effects and widespread availability compared to synthetic alternatives.[9]

The demand for natural cosmetic products with minimal side effects continues to increase. [9,10] CA is a medicinal plant known for its various therapeutic and dermatological benefits, especially in skin care and facial care.[11] This plant

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shows potent anti-inflammatory and antioxidant properties, essential in preventing photoaging caused by UVB exposure.[12,13] Previous studies have shown that topical anti-photoaging cream formulations offer better stability, solubility, and absorption, effectively preventing and treating photoaging.[14] In addition, topical and oral administration of CA has been shown to improve skin inflammation, such as atopic dermatitis caused by dinitrochlorobenzene (DNCB).[13]

The cream formulation was selected due to its ease of application, suitability for topical use, and relatively simple manufacturing process. The concentration of CA leaf extract was determined based on previous studies, which identified the 10% formulation as the most effective in enhancing collagen production over 4 weeks.[11] While prior research utilized a 4-week treatment duration to assess the effects of the 10% cream, this study employed a shorter 5-day treatment period. The 20% concentration has not been extensively compared to the 10% formulation, particularly regarding potential side effects or diminished efficacy at higher doses. Therefore, this study aims to evaluate the effects of CA extract cream at 10% and 20% concentrations over a 5-day treatment period, focusing on its potential to inhibit MMP-1 activity and enhance collagen production.

METHOD

Study Design

This study is an experimental research with a post-test-only control group design.[15]

Study Location

The study was conducted over 30 days in February 2024 at the IBL Laboratory, Faculty of Medicine, Sultan Agung Islamic University, Semarang, Central Java, and the Anatomical Pathology Laboratory, Sebelas Maret University, Surakarta, Central Java.

Preparation of CA Leaf Extract Cream

Dried CA leaves were blended, sieved, and macerated with 96% ethanol for 3 days, then re-macerated for 5 days. The filtrate evaporated in a water bath until nearly dry, weighed, and stored at -4°C. For cream preparation, the oil and water phases were weighed and heated separately to 70-75°C. The oil phase was gradually added to the water phase while stirring to form an emulsion. CA extract (10% and 20%) was then incorporated into separate mixtures and stirred until homogeneous.

Tools and Materials

The equipment for the animal model included UVB light (Lightsources FS 72T12-UVB-HO), TL-shaped lamps, razors, exposure and maintenance cages, water dispensers, and hair trimmers. Immunohistochemical tools comprised a microtome, microwave, pressure cooker, autoclave, water bath, and light microscope.[17] Histopathological analysis required slide storage, a tissue oven, slide clamps, a microscope, image analysis software, a refrigerator, a rotary microtome, disposable knives, a 60°C water bath, and mounting supplies. Materials included a cream base, CA extract, skin tissue samples in formalin and paraffin, primary and secondary antibodies for MMP-1, and detection reagents. Additional reagents were 10% formalin for fixation, alcohol (70%, 96%, absolute) for dehydration, paraffin for embedding, xylene for clearing, and Sirius Red stain for tissue staining.[1,18]

In Vivo Procedure

Dose Determination

The topical application of CA extract cream in this study used 10% and 20% concentrations. Each concentration was applied daily in an amount of 0.3 grams per mouse.[19]

UVB Exposure and Topical Application of CA Leaf Extract Cream

UVB exposure was conducted for 5 days, with daily exposure lasting 6 minutes at 1 Minimal Erythema Dose (MED)/day or 360 mJ/cm².[20] The total dose over 5 days was 1800 mJ/cm², with the UVB light positioned 30 cm from the mice's dorsal skin. After UVB exposure, the mice's skin was treated with 0.3 grams of cream.

Experimental Procedure

The study included four groups of six mice each: a healthy control (no UVB or cream) (KN), a negative control (UVB + cream base) (K-), treatment group 1 (UVB + 10% CA cream) (P1), and treatment group 2 (UVB + 20% CA cream) (P2). Mice were exposed to UVB for 6 minutes daily (1 MED/day, 360 mJ/cm²) over five days, totaling 800 mJ/cm², with the UVB lamp positioned 30cm from the dorsal skin. After each exposure, 0.3 grams of cream was applied. On day six, skin samples were collected for analysis.

MMP-1 Examination Procedure

MMP-1 inhibition in this study was assessed from dorsal skin tissue samples collected on the sixth day. MMP-1 expression was evaluated using immunohistochemistry (IHC) with a primary antibody kit (PAA097Ra01 Polyclonal Antibody to Matrix Metalloproteinase 1 (MMP-1), rabbit polyclonal, from Cloud-Clone Corp) and a secondary antibody kit (Starr Trek Universal HRP Detection). A reduction in MMP-1 expression indicated MMP-1 inhibition.

Collagen Examination Procedure

Collagen enhancement in this study was examined from dorsal skin tissue samples taken on the sixth day. A histopathological examination assessed collagen density enhancement with Sirius Red staining. An increase in collagen density was marked by a visible increase in the red colour intensity in areas containing collagen. Collagen density was measured histopathologically using an Olympus CX21 microscope equipped with an Optilab camera at 100x magnification. The images obtained were then processed using ImageJ software to determine collagen density.

Data Analysis

The research results were analyzed using statistical tests with SPSS software. A One-Way ANOVA was conducted to determine differences in MMP-1 expression among the groups, and a Kruskal-Wallis test was performed to assess differences in collagen density among the treatment groups.

Ethical Considerations

The experimental design and animal handling procedures were reviewed and approved by the Bioethics Committee of the Faculty of Medicine, Universitas Islam Sultan Agung Semarang (No. 32/I/2024/Komisi Bioetik), issued on January 30, 2024.

RESULTS

Average Results of MMP-1 Expression

The research results showed that the highest MMP-1 expression was in the negative control group (K-), followed by a decrease in P2, with the lowest in P1 (Table 1 Statistical analysis (One-Way ANOVA, p=0.053) indicated an insignificant reduction in MMP-1 expression after CA cream application (Figure 1).

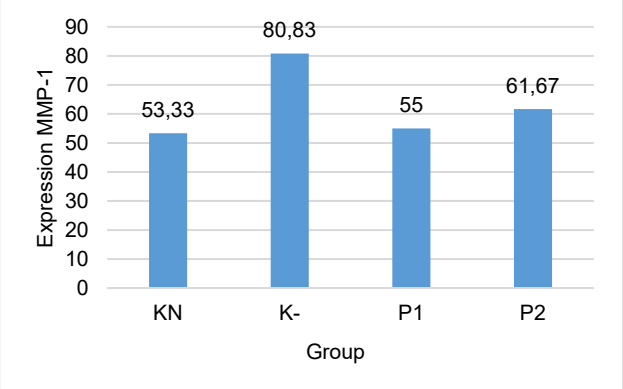

Figure 1. Mean of MMP-1 Expression

IHC analysis showed a decrease in MMP-1 expression. Positive expression appeared as brown cytoplasm, while negative expression was blue (Figure 2). Black arrows indicate positive staining. MMP-1 IHC staining was observed in approximately 50% of the epidermal cytoplasm in the KN group, 90% in K2, 40% in K3, and 60% in K4. Based on the results of the study, there was a significant difference in collagen density with the Kruskal Wallis test p=0.04. The average increase in collagen density occurred in the groups that were smeared with 10% and 20% CA leaf extract cream.

Average Collagen Density Results

The highest average collagen density results were in group (KN) and the lowest in group (K-) (Table 1). Based on the results of the study, there was a significant difference in collagen density with the Kruskal Wallis test p=0.04. The average increase in collagen density occurred in the groups that were smeared with 10% and 20% CA extract cream (Figure 3).

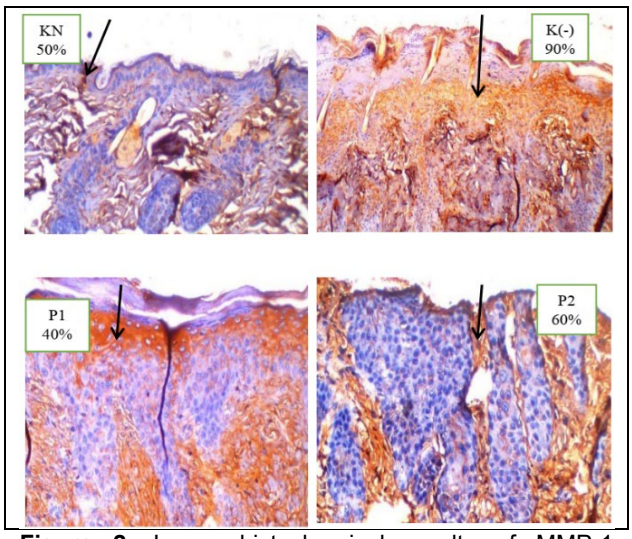

Figure 2. Immunohistochemical results of MMP-1 expression with 200x magnification

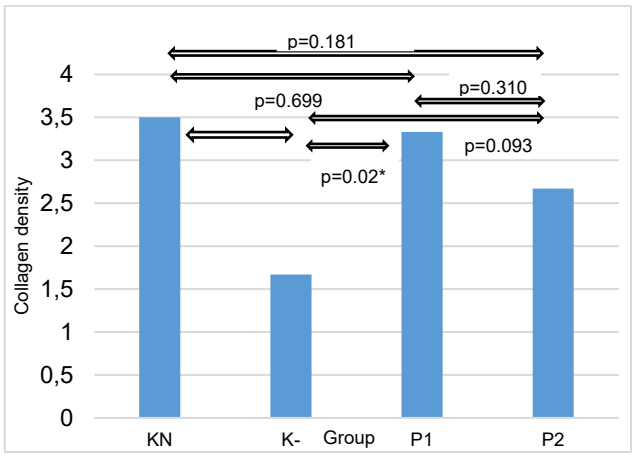

Figure 3. Average Collagen Density

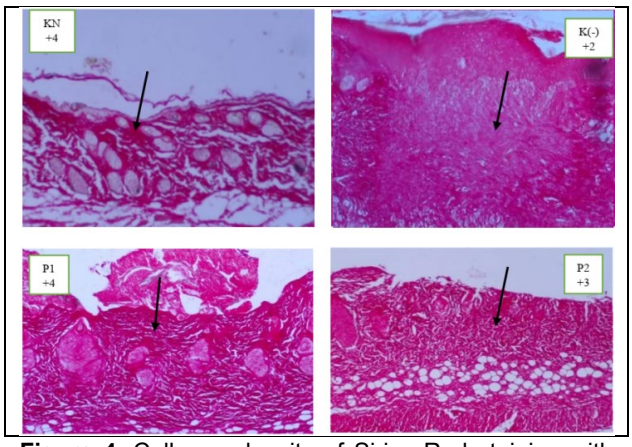

Figure 4. Collagen density of Sirius Red staining with 100x magnification

The results of the collagen density analysis were measured through histopathological examination with Sirius Red staining. An increase in collagen is indicated by an increase in the density or intensity of the red color in the area containing collagen as in the black arrow in Figure 4. The black arrow is the positive area of collagen fibers, indicated by the pink color of the fibers. KN+4 indicates that the density of collagen fibers in the wound area is very dense. K(-)+2 indicates that the density of collagen fibers in the-

Table 1. Research Results of MMP-1 Expression and Collagen Density

Variable	KN	K-	P1	P2	p-value
MMP-1 Expression	53.33±24.221	80.83±13.571	55.00±12.247	61.67±18.348	0.053*
Collagen Density	3.50±0.548	1.67±0.516	3.33±0.516	2.67±1.033	0.04**

Exp: * One Way Anova test; ** Kruskal Wallis test.

wound area is moderate. P1+4 indicates that the density of collagen fibers in the wound area is very dense. P2+3 indicates that the density of collagen fibers in the wound area is dense

DISCUSSION

This study's findings are that the administration of CA extract cream can increase collagen density but does not reduce MMP-1 expression. Increased collagen density when the skin is exposed to UVB rays does not only result from inhibition of MMP-1. Other pathways also affect collagen density, so there is a significant increase. Histological assessment of collagen density with Sirius Red staining in skin tissue of mice exposed to UVB decreased compared to normal mice. This shows that the ROS factor due to UVB exposure plays a role in collagen damage in mouse skin, oxidative stress, and impaired cellular function, including fibroblast cells that support collagen tissue.[21] After applying CA leaf extract cream, collagen density increased again. This shows that the phytochemical components contained in CA work optimally as antioxidants and anti-inflammatories.[22,23]

MMP-1 expression is influenced by many factors, such as short research time, which means that it cannot assess MMP-1 expression meaningfully, genetic variations in the DNA repair pathway, or cell stress responses that have differences in MMP-1 expression.[24] Individual genetic variations in mice produce different responses when exposed to UVB, affecting the results of MMP-1 expression and involving several genetic mechanisms. [25,26] In the CA group 10% gave more significant results than the 20% dose. This shows that a higher dose does not always mean a better effect.

Factors such as cream absorption, bioactive mechanisms in the cream, and the potential for saturation effects on biological targets may play a role. Higher doses do not always increase the therapeutic effect after reaching a certain point due to limitations in the absorption mechanism or because the target cells are already saturated with the active components. In addition, the bioactive components in CA leaf extract, such as Polyphenols, Flavonoids, Carotene, Tannin, vitamin C, and Triterpenoids, may have optimal effects at specific concentrations and are not linear with increasing doses.

In this study, it turned out that increasing the dose of CA cream to 20% did not produce better results in increasing collagen density than CA cream 10%. These results are in line with previous research on CA leaf extract cream, which reported that administering 10% CA extract cream provided the best collagen increasing effect, and in this study, the 10% CA leaf cream dose was still the best for providing a Collagen increasing effect on skin exposed to UVB.[11] CA leaf extract contains various bioactive components such as Polyphenols, Flavonoids, Carotene, Tannin, vitamin C, and Triterpenoids such as Asiaticoside, Madecassoside, Asiatic acid, Madecassic acid, which are efficacious as antioxidants, anti-inflammatory and prevent DNA damage caused by oxidative stress conditions due to increased ROS levels induced by UVB exposure. [27,28]

he antioxidant and anti-inflammatory effects help reduce damage caused by free radicals and inflammatory processes, which are important factors in the skin's photoaging process and collagen degradation.[10,29,30] Thus, the phytochemical content in CA leaves inhibits MMP-1 activity, which plays a role in collagen degradation and supports maintaining skin structural integrity. With the exposure of UVB rays to the skin of mice, further research is needed on the effect of CA extract cream on other genetic mechanisms that affect MMP-1 expression and other mechanisms besides MMP-1 inhibition related to increased collagen.[6,31]

Methodological limitations in the study, such as the location of MMP-1 preparation sampling that is not right at the wound site, can also affect the results obtained in the examination of MMP-1 expression, which is not optimal as expected. In terms of the IHC method as the choice of MMP-1 examination method in this study, it is good because it allows direct visualization of protein localization in tissue. However, other methods can provide more in-depth information, such as Real-Time Polymerase Chain Reaction (RT-PCR) or Western Blot, which can provide information about the expression and regulation of MMP-1 at the genetic and protein levels.

CONCLUSIONS AND RECOMMENDATION

Topical administration of CA extract cream improvements in decreased MMP-1 expression and significantly increased collagen density in the skin tissue of mice exposed to UVB light. Further research is needed for other parameters that increase oxidative stress levels, such as decreased levels of Superoxide dismutase and glutathione Peroxidase. The study can be continued in the clinical phase to determine the effect of CA extract cream on human skin.

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