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Application of Arabica Coffee (*Coffea arabica*) Leaf Ethanol Extract Gel Reduces Expression of Matrix Metalloproteinase-1 and Increases the Amount of Collagen in Male Wistar Rats Exposed to Ultraviolet B Light

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ABSTRACT

Introduction: This study aims to determine the effect of arabica coffee leaf ethanol extract gel on decreasing MMP-1 expression and increasing the amount of collagen in the skin of male Wistar rats (Strains) exposed to UV-B light.
Methods: This is a post-test only control group design experimental study using male rats as subjects. Each group was exposed to UV light and smeared with 5% coffee leaf ethanol extract cream every day. Then the back skin tissue was taken and the amount of collagen was examined by Picro Sirius Red staining and MMP1 levels were examined by ELISA staining.
Results: The mean MMP-1 levels in groups N, K, P1, P2, and P3 were 1.023±0.018 ng/ml, 1.578±0.28 ng/ml, 1.001±0.027 ng/ml, 0.775±0.042 ng/ml, and 0.595±0.076 ng/ml. The average percentage of collagen in groups N, K, P1, P2, and P3 was 75.63 ± 2.92%, 66.43 ± 1.67%, 72.83 ± 3.13%, 76.02 ± 3 .78, and 80.43 ± 2.12%. MMP-1 levels decreased with increasing doses of arabica coffee leaf extract gel. The mean percentage of collagen increased with increasing doses of arabica coffee leaf extract gel in experimental animals.
Conclusion: Arabica coffee leaf ethanol extract gel inhibited the increase in MMP-1 levels in male rats exposed to UV-B light. Administration of arabica coffee leaf ethanol extract gel can increase the amount of collagen in male rats exposed to UV-B light.

Keywords: Arabica coffee, metalloprtoeinase-1, collagen, UVB

INTRODUCTION

Skin aging is a very complex biological phenomenon that is controlled by many intrinsic and extrinsic factors. Exposure to ultraviolet radiation (UV) from sunlight is one of the external factors that is difficult to avoid. [1] Ultraviolet radiation is harmful to human skin because it can cause various acute and chronic negative effects on human skin such as erythema, inflammation, skin cancer, and premature aging. [2]
UVB is the most harmful constituent of UV radiation and causes photosensitive reactions and damage at the molecular level, most of which are caused by excessive reactive

oxygen species (ROS) produced in the epidermis. ROS then induce the secretion of metalloproteinase families (MMPs), such as MMP-1, -2, -3, -9, and -13, in skin fibroblasts and keratinocytes, which in turn degrade collagen and other extracellular matrix (ECM) proteins, impairing synthesis. collagen, and leads to the formation of wrinkles and photoaging of the skin. [3]

The use of cosmetic products to prevent premature aging is increasing along with technological developments and individual awareness to look attractive. Arabica coffee (*Coffea arabica)* is a natural ingredient that contains active compounds as antioxidants. Research that was previously conducted by Ermawati, (2020) regarding administration of robusta coffee extract gel can reduce the number of macrophage cells and lymphocytes. [4] The ethanol extract of arabica coffee fruit made in the form of a gel formulation has antiaging activity. Antiaging mechanism by repairing dead skin cells through changing the expression of MMP and IL-1β proteins. [5]

METHODS

This study was an experimental post-test only control group design study using male rats, aged 10-12 weeks, with body weight of 160-180 grams. The materials used in this study were 0.3% ethanol extract of coffee leaves (Coffea Arabica), aquabides, ketamine, 10% formalin, Picro Sirius Red dye. The sample was divided into 5 groups, namely the neutral group, the control group (P1) which received UV light exposure and placebo, the treatment group (P2) which received UV light and 2.5% ethanol extract gel, the treatment group (P3) which received UV light. and 5% ethanol extract gel, and the treatment group (P4) was given UV light and 7.5% ethanol gel. Each group was exposed to UV light three times a week for four weeks. The samples in the treatment group were smeared with 5% coffee leaf ethanol extract every day. Then the back skin tissue was taken and the amount of collagen was examined by Picro Sirius Red staining and MMP1 levels were examined by ELISA staining. Data is recorded and analyzed.

RESULTS

The subjects used in this study were 30 rats which were divided into 5 groups, 6 rats each, we divided the subjects to the normal control group (N), negative control (K) which were exposed to UVB light and given placebo, the other three groups were the treatment group. (P1, P2, P3) respectively exposed to UV light and arabica coffee leaf extract gel with a percentage of 2.5% (P1), 5% (P2), and 7.5% (P3). The variables observed in this study were MMP-1 levels and the percentage of collagen density.

MMP-1 levels and collagen percentage were observed after treatment on day 28. The results of the descriptive analysis of MMP-1 levels and collagen percentage after treatment for each group are presented in Table 1.

Table 1. Descriptive Analysis of MMP-1 Levels and Collagen Percentage

	Normal Control(N)	Negative Control(K)	UVB + Gel 2,5%(P1)	UVB + Gel5% (P2)	UVB + Gel7,5% (P3)
Total sampleMMP-1 level (ng/ml)	6	6	6	6	6
Minimum value	1,005	1,224	0,974	0,724	0,505
Maximum value	1,041	1,829	1,046	0,832	0,689
Mean	1,023	1,578	1,001	0,775	0,595
SD	0,018	0,280	0,027	0,042	0,076
Std. Error of	0,007	0,110	0,011	0,017	0,031
Mean					
Collagen (%) Minimum value	72,90	64,30	69,60	71,60	77,20
Maximum value	79,50	68,90	76,80	81,40	83,70
Mean	75,63	66,43	72,83	76,02	80,43
SD	2,92	1,67	3,13	3,78	2,12
Std. Error of Mean	1,19	0,68	1,28	1,54	0,87

The normality of MMP-1 level data and the percentage of collagen were tested using the Shapiro-Wilk test. The results of the data normality test are shown in Table 2. Data on MMP-1 levels were not normally distributed in the normal group (p <0.05). Data transformation with logarithms, 1/square root, and reciprocal has been done but failed to make the data distribution normal. Meanwhile, the collagen percentage data was normally distributed (p > 0.05) in all treatment groups.

Table 3. Comparison of MMP-1 Levels and Collagen Percentage between Groups

Normal Control(N)		Negative Control(K)	UVB + Gel2,5% (P1)	UVB + Gel 5%(P2)	UVB + Gel 7,5%(P3)
MMP-1 levels (*Kruskal-Wallis*) Total samples 6		6	6	6	6
Mean 1,023		1,578	1,001	0,775	0,595
SD 0,018		0,28	0,027	0,042	0,076
p-value			<0,001		
Collagen Percentage (*One Way Anova*) Total Sample	6	6	6	6	6
Mean	75,63	66,43	72,83	76,02	80,43
SD	2,92	1,67	3,13	3,78	2,12
p-value			<0,001		
Homogenitas			P=0,16		

Post Hoc test was carried out to determine the comparison of MMP-1 levels and the percentage of collagen between groups. Post Hoc test for MMP-1 levels using LSD (Least Significance Different), and Post Hoc test for collagen percentage using Bonferroni Post Hoc test results for MMP-1 levels between groups are presented in graphical form in Figure 1 and Figure 2.

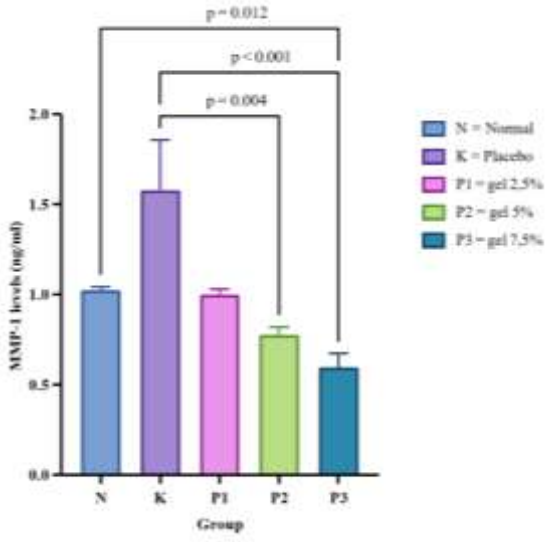

Figure 1. Graph of Comparison of MMP-1 Levels between Groups

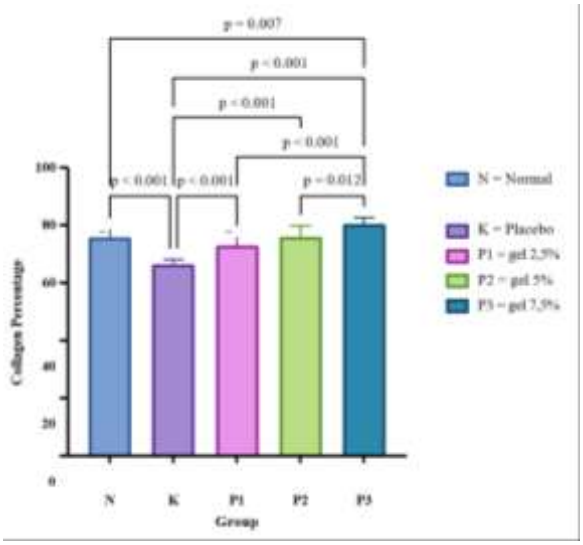

Figure 2. Graph of Comparison of Collagen Levels between Groups

Comparison of MMP-1 levels between groups showed that there were three pairs of groups that had significant differences (p<0.05), namely group N with P3, group K with P2, and group K with P3. As for the differences in other group pairs (attachment), it was not statistically significant (p>0.05). The mean MMP-1 level in group K (UVB + placebo) was higher than the normal control (N) indicating that the UVB exposure given in this study was sufficient to increase MMP-1 levels. The increase in the concentration of Arabica coffee extract in the gel was directly proportional to the decrease in MMP-1 levels.

Comparison of the percentage of collagen between groups showed that there were seven pairs of groups that had significant differences (p <0.05), namely group N with K, N with P2, K with P1, K with P2, K with

P3, P1 with P3, and P2 with P3. As for the differences in other group pairs (attachment), it was not statistically significant (p>0.05). Histopathological examination of the tissue obtained is shown in Figure 5.3 showing an image of collagen in the dermis tissue of rats observed through a microscope with 40x magnification and collagen will appear red after Pico Sirus Red staining.

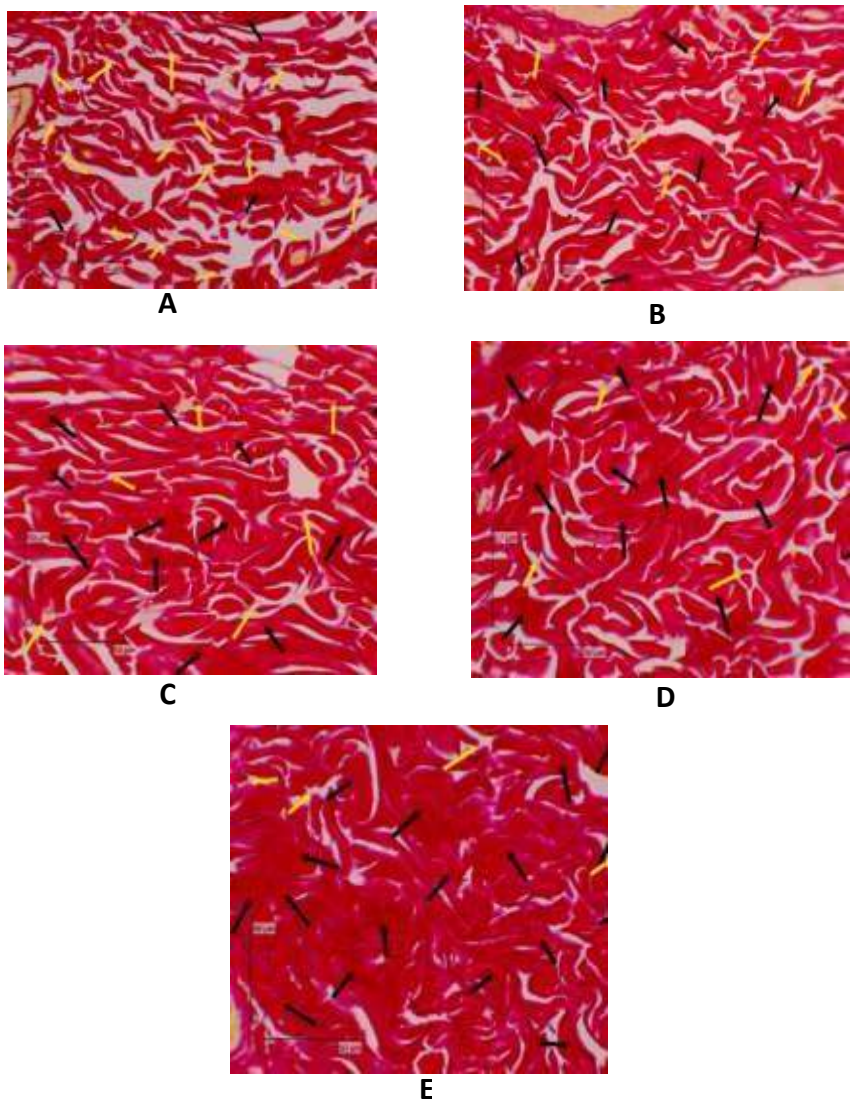

Figure 3 Collagen Experiment on Dermis Tissue with Picro Sirius Red Staining (Total Magnification 400x). The black arrows indicate intact collagen fibers. The yellow arrows indicate incomplete collagen fibers

DISCUSSION

This study used white rats as subjects because they have several beneficial properties including fast breeding, having a larger size compared to mice, easy to maintain in large numbers. The mean MMP-1 levels in groups N, K, P1, P2, and P3 were 1.023 ± 0.018 ng/ml, 1.578 ± 0.28 ng/ml, 1.001 ± 0.027 ng/ml, 0.775 ± 0.042 ng/ml, respectively ml, and 0.595 ± 0.076 ng/ml. The average percentage of collagen in groups N, K, P1, P2, and P3 was 75.63 ± 2.92%, 66.43 ± 1.67%, 72.83 ± 3.13%, 76 .02 ± 3.78, and 80.43 ± 2.12%. The decrease in MMPs expression was due to the inhibition of MAP kinase activation. The content of coffee, namely polyphenols (caffeic acid and chlorogenic acid) inhibits the activation of MAP kinase. Polyphenols inhibit the expression of ERK, JNK and p38 (which modulates c-Fos expression) so that they can suppress c-Fos and c-Jun expression and then

inhibit AP-1 expression, so that they can reduce MMPs expression and collagen degradation does not occur. Thus, the inhibition of ROS production will prevent the skin from photoaging.[6]

Antioxidants are useful for reducing free radicals which are the cause of skin aging. Antioxidants work by suppressing the formation of ROS and also work on the mitogen-activated phosphokinase (MAPK) pathway which will have implications for inhibiting the increase in MMP-1 expression. In addition, antioxidants are also rich in probiotics which have anti-aging properties in inhibiting increased MMP-1 expression through several mechanisms, namely: as antioxidants, blocking MMP-1 gene transcription through inhibition of MAPK signals; and inhibition of TNF-α.5-8 activity.[7,8]

The mean MMP-1 level in group K (UVB + placebo) was higher than the normal control (N) indicating that the UVB exposure given in this study was sufficient to increase MMP-1 levels. The increase in the concentration of Arabica coffee extract in the gel was directly proportional to the decrease in MMP-1 levels.

In coffee there are flavonoid compounds as antioxidants, although it is also explained that these flavonoids are not enzymatic or non-enzymatic antioxidants. Flavonoid compounds can transfer hydrogen electrons[9,10]. However, free radicals that have been proven to be neutralized by flavonoids cannot be proven.[11] Protects the skin from oxidative damage induced by ultraviolet exposure because the antioxidant compounds in the body are quite high. Antioxidants can inhibit the formation of ROS, and further inhibit collagen degradation by UVB light exposure and increase the amount of dermal collagen. [8,12]

Coffee has the ability to increase the production of collagen and elastin which are useful for providing protection against reduced moisture.[13] This is because coffee contains chlorogenic acid which is a potent antioxidant from phenolic compounds that can inhibit oxidative damage so that it can have an antioxidant effect.[14,15]

CONCLUSION

Arabica coffee leaf ethanol extract gel inhibited the increase in MMP-1 levels in Wistar male rats (strains) exposed to UV-B light. Administration of arabica coffee leaf ethanol extract gel can increase the amount of collagen in male Wistar rats (strains) exposed to UV-B light.

Declaration by Authors

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