Egyptian Journal of Basic and Clinical Pharmacology
Vol. 9 (2019), Article ID 101408, 6 pages
doi:10.32527/2019/101408

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Research Article

Effects of Klika Faloak (*Sterculia Populifolia*) Extract Cream Toward MMP-1 Expression of Albino Mice Against Ultraviolet B Radiation

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Dates: Received 29 November 2018; Accepted 27 January 2019

Editor: Abdel-Aziz H. Abdel-Aziz

Abstract. Klika Faloak (*Sterculia Populifolia)* extract contains flavonoid and polyphenol compounds which are the most potential components in reducing ROS (Radical Oxidative Superoxide) to prevent free radical caused by UV-B radiation. This study aimed to prove that Klika Faloak extract cream could reduce the expression of matrix metalloproteinase-1 (MMP-1) in albino mice exposed to UV-B radiation. This study was a laboratory experimental study using randomized pre and post-test design. Albino mice were divided into 3 groups; group 1 was treated with 0.1 mg/cm^2 on 3 cm^2 of radiation area with 5% Klika Faloak extract cream for four weeks and exposed to UVB radiation, group 2 was treated with 0.1 mg/cm^2 on 3 cm^2 of radiation area with base cream/ placebo for four weeks and exposed to UVB radiation, and group 3 was controlled group which not given anything and not exposed to UVB radiation. Prior to the treatment, MMP-1 expression was examined through blood serum and then the treatment group was exposed to UV-B radiation at a dose of 500 mJ/cm^2 for 4 weeks. After that skin biopsy was performed to examine MMP-1 expression. Data were analyzed using one way ANOVA to determine significant difference among three groups then followed by a post hoc test using the LSD (Least Significance Difference) to determine the smallest significant level <0.05. The result showed average decreasing of MMP-1 expression in group 1 compared to group 2 and group 3. It could be concluded that Klika Faloak extract cream could reduce MMP-1 expression.

Keywords: Klika Faloak, Extract Cream, UV-B, Matrix Metalloproteinase-1, Radical Oxidative Superoxide.

1. Introduction

Skin aging involves intrinsic and extrinsic process. Environmental factors, especially ultraviolet (UV) radiation causes extrinsic skin aging. Overexposure to UV radiation, particularly to UVB (290–320 nm), leads to skin damaged called photo aging which is characterized by wrinkle formation, dry and rough skin, irregular pigmentation and poor elastic recoil [1]. Chronic exposure to UV radiation leads to overproduction of reactive oxygen species (ROS) in epidermis which could destroy anti-oxidant defense system in the body and finally cause oxidative stress [2–4]. Oxidative stress induces formation of lipid peroxidation, through lipid-derived radical, which leads to disruption of cell membrane, and consequently cell death [5]. In addition, overproduction of ROS triggers the release of pro-inflammatory cytokines such as interleukin (IL)-1, -6, -8 and tumor necrosis factor-alpha (TNF-α) from keratinocytes of the epidermis [6]. In the fibroblasts of dermis, these cytokines induce expression of matrix metalloproteinase (MMPs) which play key roles

in degradation of collagen, elastin and other proteins in connective tissue and bone [7].

The MMP-1 is an enzyme that contributes to degradation of collagen in the skin that has photo-aging. MMP-1 enzyme in the skin would increase even in a short UV exposure then it would cause wrinkles as a sign of photo-aging [8]. In human skin, MMP-1 is the most influenced enzyme by UV induction of sun and is responsible for collagen degradation of skin undergoing photo-aging [9]. It was found that there was an increase in MMP-1 levels through gene expression regulation that involving significant histone modification or epigenetic regulation compared to cell cultures which were not exposed to UV radiation [10]. Thus the detention to MMP-1 is the way to prevent skin damaged due to UV exposure.

Plants are potential source of natural antioxidants. Natural antioxidants or phytochemical antioxidants are the secondary metabolites of plants [11]. Carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols, tocotrienols etc., are some antioxidants produced by plant for their sustenance. Beta-carotene, ascorbic acid and alpha tocopherol are widely used antioxidants [12]. Klika Faloak (*Sterculia populifolia)* is mostly found in Timor Island, East Nusa Tenggara Province (NTT). The people of East Nusa Tenggara Province use Klika Faloak as a traditional medicine based on hereditary knowledge and experience [13]. Klika Faloak contains a number of antioxidants such as ascorbic acid, terpenoids, alkaloids, polyphenols such as flavonoids, flavone glycosides, rutin etc [14]. Easily cultivable Klika Faloak with its wide range of antioxidants can be a major source of natural or phytochemical antioxidants.

2. Material and Method

2.1. Materials and tools. The steam bark of Klika Faloak (c.a 10 kg) was collected from a local area in Kupang, east Nusa Tenggara, Indonesia in November 2015. The identification of plant was done by Herbarium-LIPI, Purwodadi, East Java, Indonesia. The ingredients of the formulation cream were consisting of petrolatum, mineral oil and isopropyl myristate which obtained from Merck (German), stearic acid and glycerol monostrearate from Franken Chemical (German), and triethanolamine, nipagin, nipasol and xanthan gum from Wacker Chemicals (German).

The equipment used to do extraction was filter paper, Erlenmeyer flask, pipette, separating funnel and vacuum rotary evaporator type BUCHI Rota vapor. Equipment used to make creams was homogenizer, Erlenmeyer, beaker, hot plate, and thermometer.

3. Procedure

3.1. Extraction of Klika Faloak. The fresh stem bark of Klika Faloak (c.a 10 kg) was washed under water tap, dried under sunlight for seven days and then dried in an oven at temperature of no more than 50˚C to make it suitable for grinding purpose. It was ground using electric grinder (3.8 kg) and moved to air-tight container. The powder stem bark (3.8 kg) was macerated using ethanol 70% (c.a 13 L) at room temperature. The container with its content was sealed by foil and kept for a period of 24 h accompanying occasional shaking and stirring for three times. The whole mixture was then filtered using Buchner funnel and the filtrate was concentrated at 50˚C with a vacuum rotary evaporator and freeze dried for 24 hours. The concentrated extract obtained is called as brown crude extract (76 g).

3.2. The making of Klika Faloak extract cream. The base formula O/W, modified by Bernatoinene et al, was petrolatum (6.2 g), mineral oil (13.8 g), isopropyl myristate (1.5 g), stearate acid (7.5 g), glycerol monostearate (5 g), nipasol (0.05 g), TEA (0.2 g), xanthan gum (0.2 g), nipagin (0.1 g), Klika Faloak extract 5%, and aquadestilata (ad 100 g) [15].

The oil phase which composed of petrolatum, mineral oil, isopropyl myristate, strearate acid, glycerol monostearate and nipasol were heated until 70˚ degrees Celsius. The water phase which consisted of TEA, xanthan gum, nipagin and aquadestilata were also heated in 70˚ degrees Celsius. The cream was made by adding the water phase to the oil phase carefully. Then, the manual stirring was done constantly in an anticlockwise direction to lower the temperature until 35˚ degrees Celsius. Last but not least, 5% of Klika Faloak extract was poured into the base cream and crushed to homogeneous.

4. Animal

This study was using 15 mice range in age 6–8 weeks with the average weight of 20–30 gr which obtained from Animal Laboratory of Padjajaran University, West Java, Indonesia. The animal experimental was approved by the institutional ethics committee for animal care of Hasanuddin University and was conducted in accordance with the guidelines for the care and use of laboratory animals (Institute of Laboratory Resource, 1996) as adopted and promulgated by the National Institutes of Health.

5. Research Procedure

For the experiment, 15 albino mice were randomly divided into three groups. Mice in group 1 were given Klika Faloak extract, base cream for mice in group 2, and mice in group 3 were given no radiation. An adjustment was given to mice in order to adapt with the laboratory environment in a week. The mice were also weighed, before the experiment started. Each mice had a blood sample taken to be examined for mRNA MMP-1 expression. Then, each mice should be shaved on its back for radiation area. The radiation was done using Kernel UV B stimulator type KN-4003B (Philips, Somerste,

Cina) with an emission spectrum between 275 and 380 nm (peak: 310–315 nm). UV-B radiation intensity on the mouse skin surface was measured using a UV meter (Waldmann GmbH&Co, Vilingen-Schwenningen, Germany). The radiation intensity at 30 cm from the light source was about 0,5 mW/cm^2. Initially, the minimal erythema dose (MED) to induce erythema with sharp margins on the dorsal skin of the mice after 48 was defined as 1 MED, which was calculated to be approximately 500 mJ/cm^2. The mice were exposed to UV light 3 time per week (Monday, Wednesday and Friday) for a total 4 weeks. The radiation dose was increased weekly by 1 MED from 1 MED up to 3 MED and then maintained at 3 MED until the end of the experimental.

After that, the Klika Faloak extract cream for group 1 and base cream for group 2 were applied twice a day for 0.1 mg/cm^2 on 3 cm^2 of radiation area in 20 minutes before the radiation. It was done to give time for the topical ingredients absorb to the skin. The Klika Faloak extract cream and base cream were also applied in 4 hours after the radiation since ROS started to form in 4 hours after the exposure. The application of topical cream should be done daily, including the day without radiation. After 4 weeks treatment, in the last radiation day, the mice should be abandoned first for 24 hours. This was done in order to remove the effect of acute radiation before getting sacrificed. Further, the biopsy skin of each mice was taken to get the mRNA MMP-1 expression examination.

6. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Each 100 µl fresh blood sample and skin were mixed into 900 µl lysis buffer L6 screw-caped tube. Then the mixture was centrifuged to 12.000 rpm for 10 minutes. The sample sediment that has been concentrated will be homogenized for 30 minutes. Before adding the diatom suspension, the buffer L6 mixture, which already contained RNA from extraction, was centrifuged for 2-3 minutes in 12.000 rpm to make the RNA from extraction settled in the bottom of the tube. The diatom suspension of 20 µl was added to the tube, and the diatom suspension must always be vortex and stirred using gyratory shaker in 100 rpm for 10 minutes. The mixture of diatom and buffer L6 vortexed back using centrifuge with micro centrifuge Eppendorf in 12.000 rpm for 15 seconds. The supernatant, which formed from each vial, was separated with sucker made of plastic Pasteur pipette without air balloon and connected to vacuum pump to prevent the disappearance of diatom in suspension, and remained the suspension for about 10 µl [16].

The supernatant was washed twice using 1 ml of washing buffer L2. The washing buffer L2 was added, vortexed and centrifuged in 12.000 rpm for 15 seconds before disposing the supernatant. The sediment was washed again twice with 1 ml of 70% ethanol, then vortexed and centrifuged in 12.000 rpm for 15 seconds and disposed the supernatant. Further, the sediment was washed again with 1 ml of acetone, vortexed and centrifuged in 12.000 rpm for 15 seconds and disposing the supernatant again. The remaining acetone in the sediment was evaporated by opening the vial cape and heated up with oven in 50–55˚ degrees Celsius for about 10 minutes. After the sediment has dried, TE buffer elusion was added for 60 ml and then vortexed evenly to make the sediment and suspension dissolved. Then, the vial was incubated in oven in 56˚ degrees Celsius for 10 minutes. Then, the mixture was centrufuged in 12.000 rpm for 30 seconds and carefully took the supernatant for 40–50 µl and put inside the new vial tube. The extraction result could be saved in –20˚ or –80˚ degrees Celsius [16].

Total RNA were isolated from serum and skin biopsy samples stored in liquid nitrogen using Triazol (invitrogen, USA) according to the manufacturer's protocol and its concentration was determined by measuring the absorbance at 260 and 280 nm. The quality and quantity of the extracted RNA were confirmed by electrophoresis in 1% denaturing agarose gel. The oligonucleotide primer sequences for RT-PCR analysis to estimate the murine m-RNA level of MMP-1 (5'GCTAACCTTTGATGCTATAACTACGA-3' and 5'-TTTGTGCATGTAGAATCTG-3') [17]. For RT-PCR reaction, 2 µg of total RNA was incubated with 50 ng of random hexamers and 200 U of reverse transcriptase (invitrogen, USA) for 50 min at 50˚C, and the reaction was terminated at 85˚c for 5 minutes. A1: 10 dilution of the reaction mixture containing c-DNA was subjected to PCR amplification using 20 pmol of primers and 1 U of amfiSure Taq DNA polymerase (Gendepot, Barker, USA) with the cycling program: 1st cycle, 15 min at 94˚C, next 32 cycles; 30 s at 94˚C, 30 s at 56˚C, and 30 s at 72˚C and the final cycle, 7 min at 72˚C. 20 µL of the PCR product was separated by electrophoresis on 11.5% agarose gel and stained with ethidium bromide.

7. Results

7.1. Age and weight of mice. The data of mice's average weight and age could be seen in the Table 1. The normality and homogeneity test of weight and age obtained $p>0.05$ that means there were no significant differences. This also showed that data were normally distributed and homogenous.

Table 1: The average weight and age of mice.

Group	Weight	P^x	Age	P^x
Group 1	16.02 ± 0.563	0.125	6.8 ± 0.447	0.146
Group 2	16.1 ± 0.561	0.125	6.6 ± 0.548	0.119
Group 3	16.12 ± 0.531	0.217	6.6 ± 0.548	0.314
	P^{xx}	0.811*	P^{xx}	0.053*

* One way ANOVA

7.2. MMP-1 expression. The analysis effect of treatment was tested using average expression of MMP-1 among groups before and after treatment. The test for before treatment group was taken through blood serum, while the skin biopsy was taken after the treatment. The blood serum was taken first in order to prevent skin damage if skin biopsy was taken first. It was assumed that blood and skin biopsy in mRNA examination had the same result as the result obtained by group 3 that there was no differentiation in MMP-1 expression between blood and skin biopsy.

Table 2 shows that group 1 had p = 0.005 that means average expression of MMP-1 before and after the treatment had significant differences (p<0.05). Group 2 obtained p = 0.002 that means the average expression of MMP-1 before and after treatment was significantly different (P<0.05), while group 3 had p = 0.586 that means the average expression of MMP-1 before and after treatment was not significantly different (p>0.05).

Table 2: Average expression of MMP-1 before and after treatment.

Group		**Average of MMP-1 Expression**	**SB**	**T**	**P**
Group 1	Before	9.309	0.811	5.618	0.005[#]
	After	6.521	1.002		
Group 2	Before	10.179	0.375	7.229	0.002[#]
	After	12.455	0.541		
Group 3	Before	9.134	0.874	0.591	0.586[#]
	After	9.288	0.613		

[#] paired-t-test

Table 3: The average expression of MMP-1 among groups before and after treatment.

Group	**Before**				**After**			
	Average of MMP-1 Expression	SB	F	P	Average of MMP-1 Expression	SB	F	P
Group1	9.309	0.811			6.521	1.002	79.007	0.000*
Group2	10.179	0.375	3.007	0.087*	12.455	0.541		
Group3	9.134	0.874			9.288	0.613		

* One way ANOVA

Table 3 shows the result of significance analysis with one way ANOVA test. The test obtained F = 3.007 and p = 0.087. This means that there was no significant difference in MMP-1 expression before treatment among groups (p> 0.05). While the result of significance test after the treatment obtained F = 79.007 and p = 0.000. It means that there was significant difference of MMP-1 expression among groups after the treatment (p<0.05).

Then it was continued with Post Hoc test, namely the Least Significant Difference-test (LES) that was used to determine the smallest significant difference of MMP-1 expression average after treatment. Test results could be seen in the Table 4.

Table 4: The LSD of MMP-1 Expression after Treatment.

Group	Average Difference	P	Interpretation
Group 1 and Group 2	5.933	0.000*	Significant Different
Group 1 and Group 3	2.766	0.000*	Significant Different
Group 2 and Group 3	3.167	0.000*	Significant Different

*Least significant difference

8. Discussion

This study used healthy and normal albino mice (aged 6–8 weeks, weighing 20–30 grams). Albino mice were used because mice are mammalian vertebrate animals whose skin structure is similar to human skin besides albino mice do not have pigments including hair follicles. While rats aged 6–8 weeks were chosen because the structure of the skin has similarities with young adult humans who have not experienced intrinsic aging. Vayalil et al, examined green tea and its effects on ultraviolet exposure, using SKH-1 mice without hair in their research. SKH-1 mice without hair

are ideal experimental animals in this study because they do not require shaving therefore they are more practical in ultraviolet light exposure [14]. The problem is strain mice without hair have not been found in Indonesia yet. The results of one way ANOVA analysis in Table 1 show that the average age and weight of mice had no significant differences in each group.

The exposure of skin by ultraviolet both directly and indirectly would cause negative effects. As many as 50% of skin damage caused by UV is estimated due to the formation of free radicals, while the remaining 50% is caused by cellular damage and other mechanisms [11]. UVB doses that could cause a decrease of MMP-1 expression in mice are varied widely. Djawad used UVB exposure at a dose of 343 mJ/cm^2 three times a week for 4 weeks [18]. Adriani used UVB exposure at a dose of 450 mJ/cm^2 three times a week for 4 weeks [19]. Referring to the study, the UVB dose used in this study was 500 mJ/cm^2 three times a week for 4 weeks.

This study obtained a significant decreased of MMP-1 expression average of group 1 before and after UVB exposure (Table 2). The degradation of MMP-1 expression after the skin radiation indicated that Klika Faloak extract could protect the skin from oxidative damage of ultraviolet exposure. According to Khairi et al, Klika Faloak extract indicated as a powerful antioxidant with score for IC_{50} 16.56 ppm. This powerful antioxidant was caused by the flavonoid and polyphenol compound which contain inside the Klika Faloak [20]. Flavonoids and polyphenols in Klika Faloak extract could prevent damage caused by free radicals in several ways, one of which is destroying free radicals directly [21]. Flavonoids are oxidized by radicals and producing more stable and less reactive radicals. Flavonoids stabilize reactive oxygen by reacting with the reactive composition of these radicals [22]. The neutralization of free radicals by flavonoids causes no cytokine receptor activity and growth factor on the surface of epidermal keratinocytes and fibroblasts in dermis. By inactivation of these receptors, MAP kinase intracellular singular pathway causes the AP-1 transcription factor to be inactive. This results the cessation of MMP-1 transcription therefore the level of MMP-1 in the skin decreases, as indicated by a decrease of mRNA MMP-1 expression [23, 24].

The polyphenol in Klika Faloak extract that was given topically could have an effect as photo-protection. The antioxidant mechanism of polyphenol is based on its ability to donate hydrogen atoms and the ability to chelate metal ions. After donating a hydrogen atom, phenolic becomes stable and not susceptible to resonance, thus they do not easily participate in other radical reactions [21, 25]. By reducing the reactivity of free radicals, the administration of these polyphenols could prevent the synthesis and accumulation of MMP in the skin.

MMP is the main mediator for the emergence of collagen degradation in the skin that experiences photo-aging. MMP-1 enzyme degrades collagen and elastin fibrils which are important for skin strength and elasticity. MMP1 activity in the skin would increase even with short UV radiation, which would cause wrinkles on the skin, which is a sign of photo-aging [8]. Thus, the resistance to MMP, especially MMP-1 is one way to prevent skin damage due to UV exposure. Oxidative stress has a major effect on the process of photo-aging and photo-carcinogenesis and also pathogenesis of photo-dermatosis [26].

In group 2, it was obtained significant increase of MMP-1 expression after UVB exposure. Increased MMP-1 expression after UVB radiation was caused by energy from UV radiation damaging cell membranes and proteins to produce reactive oxygen species (ROS), which inducing the expression of pro-inflammatory cytokines that binding to cell surface receptors including receptors of epidermal growth factor, interleukin (IL) -1, insulin keratinocyte grown factor and tumor necrosis factor (TNF). Activation of these receptors makes ROS to inhibit the protein tyrosine phosphatase enzyme, which has functions to maintain inactive epidermal growth factor receptors. The activity of kinase induces transcription of complex core of AP-1, a protein complex containing c-Jun protein and c-Fos[27]. AP-1 increases MMP gene transcription and decreases TGF-β procollagen receptor then consequently decreases dermal matrix formation. In skin, the combination of collagenase (MMP-1), 92kDa gellatinase (MMP-2), 72kDa gellatinase (MMP9) and stromelisin 1 (MMP3) could completely degrade collagen and components of elastin tissue. Even though the expression of all these enzymes is very low in normal skin, the enzyme could increase after UV exposure. There are cell cultures in vivo and in vitro [27]. It was obtained no difference in MMP-1 level of group 3 as a controlled group.

The results of one way ANOVA statistical test on the average expression of MMP-1 before UVB exposure had no difference in MMP-1 expression for all groups, while the results of statistical tests on MMP-1 expression average after UVB exposure had significant differences in MMP-1 expression for all groups. LSD statistical tests of MMP-1 expression average also had significant differences in MMP-1 expression for all groups.

9. Conclusion

Based on the results of the study, it could be concluded that the topical administration of Klika Faloak extract cream in albino mice exposed to UVB could reduce MMP-1 expression compared to groups of albino mice given placebo cream and controlled group. Thus, Klika Faloak is effective to protect the skin from UVB through reducing mmpl expression.

Competing Interests

The authors declare no competing interests.

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